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CONTRACTING ORGANIZATION: Lipitek International, Incorporated
                            San Antonio, Texas   78245

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13. ABSTRACT *(Maximum 200*

The primary goal of this Phase I study was to test novel L-nucleoside compounds for their toxicity against the malaria parasite, Plasmodium falciparum. Preliminary investigations were also made, using selected compounds, to evaluate the transport and uptake of the infected erythrocyte and the metabolism of the compound by the parasite. L-nucleoside compounds suggested themselves for this application because of their low toxicity, which has been established in previously performed anticancer screens, both in vitro and in vivo, and their unique and selective ability to be transported into an infected erythrocyte The toxicity screens yielded several compounds with very promising activity. Since the compounds are practically non-toxic, it makes these results even more exciting., The transport and metabolism tests indicated that the compounds were being taken up by the invaded cells and were being metabolized. The tests also indicated that healthy red blood cells did not take up the compounds, did not metabolize them and were unaffected by the toxicity that affected the parasitized cells.

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I. INTRODUCTION

Malaria afflicts around 200 million people annually, making it a major cause of human morbidity and mortality worldwide [1]. The most fatal form of disease is caused by the protozoan parasite *Plasmodium falciparum*. The parasite invades human red blood cells and immediately begins to remodel the cell, making both internal and external modifications, that enable the pathogens to survive and proliferate in the host. The alterations facilitate the movement of nutrients into, and waste products and parasite derived proteins out of the cell to meet the needs of the growing parasite. The mechanism of these modifications is largely unknown.

During the last decade, malaria research has been intensified at both the applied and the basic biological levels. Although most of the resources have been devoted to vaccine development, there is still no safe and effective vaccine available for malaria prevention.

The "classical" antimalarial drugs have been nucleic acid intercalators such as acridines, phenantrenes and quinolines which complex with nucleic acids and thus interfere with the parasite's ability to replicate. These are toxic drugs and the side-effects are dose-limiting. Cross-resistance between these classes of drugs is also a common therapeutic problem. An urgent need exists to develop new drugs for the therapy and prophylaxis of malaria in both the civilian and military arenas.

Recent investigations into the biochemistry of the host and parasite cells have shown significant differences between normal and infected erythrocytes with respect to purine and pyrimidine metabolism in single enzymes, as well as in whole branches of related pathways. The parasite satisfies all of its purine requirements through salvage pathways, which the host cell lacks, and meets its pyrimidines requirements principally from *de novo* synthesis [2]. These alterations in the biochemistry of infected erythrocytes versus normal healthy red blood cells may therefore be used in the rational design of new drugs that selectively inhibit transport of nutrients or their metabolism by the malaria parasite.

It has been established that the malaria infected erythrocyte is capable of effectively transporting "unnatural" L-nucleosides, whereas normal mammalian cells are nonpermeable to this class of compounds [3]. Since most of the L-nucleosides are not recognized by mammalian enzyme systems (being the stereochemical antipodes of natural D-nucleosides), they are usually not toxic. Thus, L-nucleosides or their derivatives may be used as selective cytotoxic drugs against parasite-infected cells without affecting normal erythrocytes. This is the underlying principle of this Phase I investigation.

Lipitek International is a leader in the design and synthesis of novel L-nucleoside based therapeutics. Over last three years Lipitek has established a large library of L-nucleosides and their derivatives which demonstrate low toxicity profile and novel modes of effective antiviral, anticancer and anti-parasitic activity. Lipitek has established a research liaison with the laboratory of Dr. Annette Gero at the University of New South

Wales, Australia. Dr. Gero is an internationally recognized expert in malaria research and a pioneer in the biochemistry of the nucleoside metabolism in malaria infection. This alliance enabled us to investigate a number of L-nucleosides and their derivatives in *in vitro* tests against the protozoan parasite *P. falciparum* and establish a preliminary structure-activity relationship. It was also proposed to get a preliminary insight into the metabolism, transport, and mechanism of action of selected L-nucleosides and their conjugates. The biological testing was all performed in Dr. Gero's laboratories at the University of New South Wales in Sydney, Australia.

Selected test compounds have also been submitted to the U.S. Army's Antimalarial Test Program.

II. ASSUMPTIONS

Based on the available preliminary data on selectivity of the uptake and metabolism of L-nucleosides in malaria infected cells versus normal red blood cells, the following assumptions were made :

1) Lipitek's collection of L-nucleosides and their dimers, particularly those containing 5-FUdR , will be effective inhibitors of the protozoan parasite *P. falciparum*.

2) L-nucleosides and their dimers will have low toxicity against normal cells.

3) The forty L-nucleoside analogs proposed for screening will provide information on structure-activity relationships and enable us to select one or two leads for further evaluation.

4) Preliminary data on the metabolism, transport, and mechanism of action of the tested L-nucleoside analogs will be collected.

5) The results of Phase I research will enable us to design more effective L-nucleoside conjugates for a Phase II study and will lead to the nomination of a drug candidate for development.
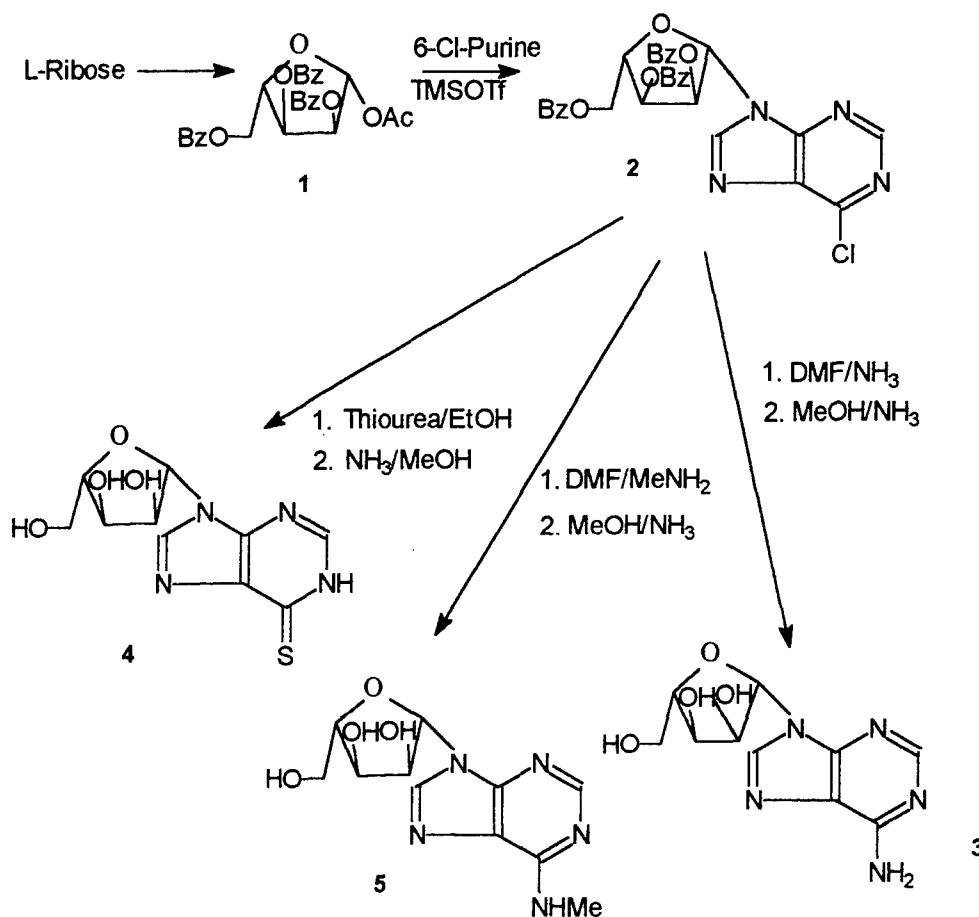
III. METHODS AND PROCEDURES

III.1 CHEMICAL SYNTHESIS

All chemical synthesis was performed at Lipitek International, Inc..

III.1.1. Synthesis of monomers

Monomers 3, 4 and 5 have been synthesized as shown in Scheme 1.

Scheme 1

9-(2,3,5-Tri-O-benzoyl-β-L-ribofuranosyl)-6-chloropurine (2)

A mixture of 6-chloropurine (17.0 g, 0.11 mol) and $(NH_4)_2SO_4$ (catalytic amount) in HMDS (200 mL) was refluxed for 8 hours. The resulting solution was concentrated under anhydrous conditions to yield silylated 6-chloropurine. To a cooled (0°C) and stirred solution of silylated 6-chloropurine and 1-O-acetyl-2,3,5-tri-O-benzoyl-β-L-ribose (1) (27.77 g, 55 mmol) in dry dichloroethane (400 mL), TMSOTf (21.2 mL, 110 mmol)

was added. The reaction mixture was warmed to room temperature and stirred for 16 hours. The reaction was quenched with saturated $NaHCO_3$ solution (25 mL) and the solvent was evaporated. The residue was dissolved in EtOAc, washed with water brine, dried, filtered and evaporated to give a solid residue. It was then purified on a silica gel column using EtOAc:PetEther (30-40%) to give pure (2) (30.8 g, 93%) as foam.

9-β-L-Ribofuranosyladenine (3)

A solution of (2) (5 g) in DME/NH_3 (200 mL) was heated at 80°C in a steel bomb for 24 hours. After cooling, the solvent was evaporated and the solid obtained was stirred in $NH_3/MeOH$ (300 mL) overnight. After the evaporation of the solvent, the residue was dissolved in water (200 mL), washed with $CHCl_3$ (2x50 mL) and ether (2x50 mL). The water layer was evaporated and the residue crystallized from water to give pure (3) (2.1 g, 93%) as white crystals (m.p. 225°C(dec)).

9-β-L-Ribofuranosyl-6-thiopurine (4)

To a solution of (2) (1.0g, 1.66 mmol) in anhydrous EtOH thiourea was added (0.25 g, 3.34 mmol). The reaction mixture was refluxed for an hour and then the solvent was evaporated. The residue dissolved in EtOAc, washed with water, and dried. After evaporation of the solvent, the crude product was triturated with EtOH to yield benzoylated thioquanine. It was debenzoylated by stirring it with $NH_3/MeOH$ (150 mL) at room temperature overnight. After evaporating the solvent, the solid obtained was dissolved in water and washed with $CHCl_3$ (3x50 mL). Then the water was concentrated and the crude product was crystallized from water to give pure (4) (0.25 g, 52%) as yellow crystals.

9-β-L-Ribofuranosyl-N^6-methyl adenine (5)

To a solution of (2) (2.0 g, 3.32 mmol) in DME (100 mL), methyl amine (3 mL) was added and the mixture was heated in a steel bomb for 5 hours at 80°C. Then the solvent was evaporated and $NH_3/MeOH$ (200 mL) was added. The reaction mixture was stirred overnight. After evaporating the solvent, the crude was purified by a silica gel column using 15-20% $MeOH/CHCl_3$ to give pure (5) as white solid (0.78 g, 83%).

III.1.2. Dinucleoside Monophosphate Dimers (DNMP)

These were prepared by the general route shown in Scheme 2. This method has been optimized at Lipitek International and used successfully in the preparation of a large variety of L-nucleoside-containing dimers. The individual nucleoside monomer components utilized in the synthesis were either L- or D-nucleosides. α-L and β-L isomers of 5-FUdR, deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine have already been synthesized at Lipitek and used as building blocks for the synthesis.

Scheme 2

A)

B)

C)
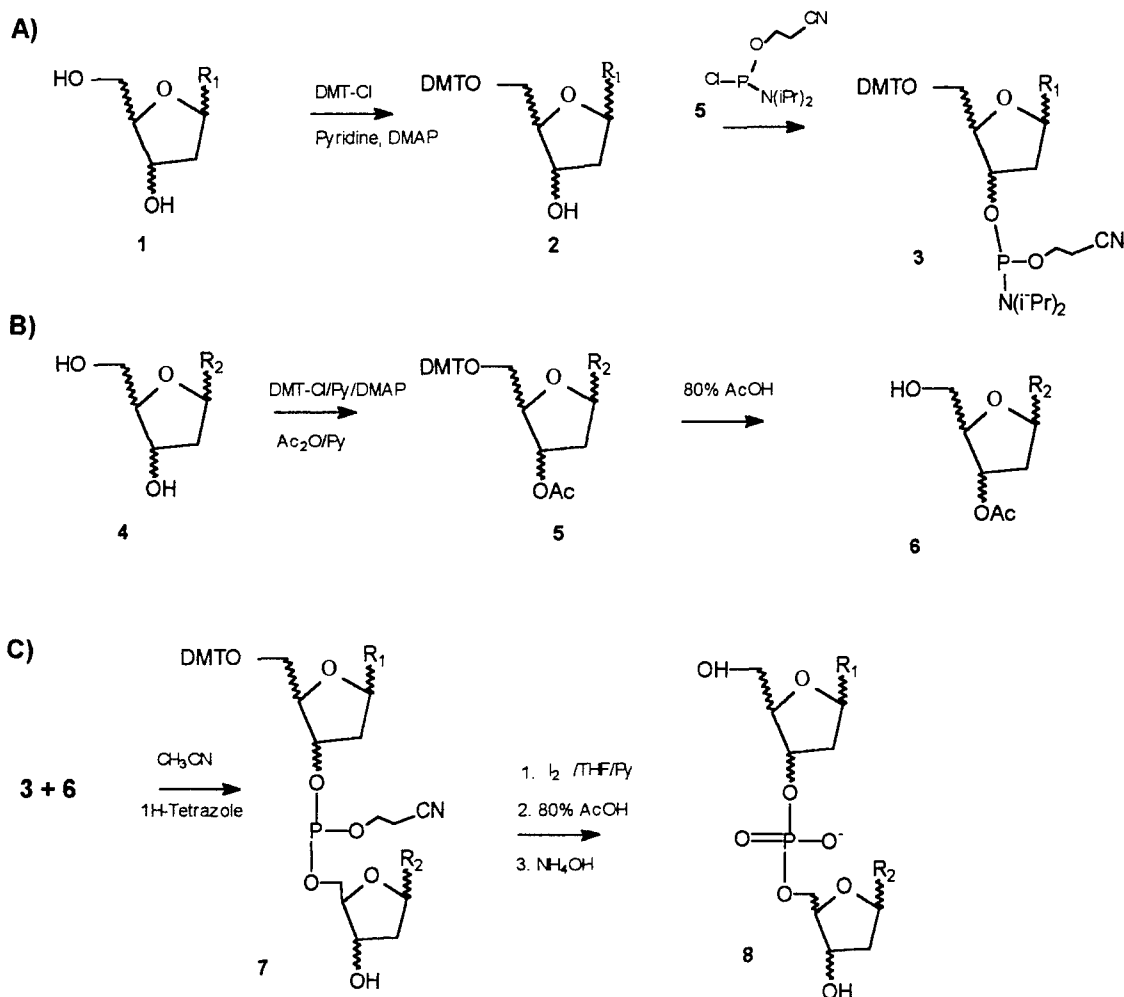
5'-O-Dimethoxytrityl nucleoside (2)

The deoxy nucleoside **(1)** (1 mmol) was dissolved in pyridine (5 mL). To this solution 4,4'-dimethoxytrityl chloride (1.2 mmol) and 4-dimethylamino pyridine (0.3 mmol) were added. The mixture was stirred under argon atmosphere for 3 h. Then the pyridine was evaporated and the crude was dissolved in EtOAc. The ethyl acetate solution was washed with water, NaHCO$_3$ and brine. The solvent was evaporated and crude was purified by flash chromatography using MeOH / CHCl$_3$ (3:97 %) as eluent to obtain pure **(2)**.

Phosphoramidite (3)

To a solution of **(2)** (1 mmol) N,N-diisopropylethylamine (4 mmol) was added followed by the addition of 2'-cyanoethyl-N,N-diisopropyl chlorophosphoramidite (1.3 mmol). The reaction mixture was stirred for 30 min under argon and then the anhydrous methanol (0.2 mL) was added to quench the reaction. The solvent was evaporated and the crude was redissolved in 80 : 20 EtOAc / Et$_3$N mixture. The solution was washed

with water, NaHCO₃ and brine. The solvent was evaporated and the crude was purified by silica gel chromatography using mixture of 50:40:10 EtOAc:CH₂Cl₂:Et₃N as eluent to obtain pure **(3)**.

3'-Acetyl nucleoside (5)

To a solution of **(4)** (1 mmol) in pyridine (5-10 mL) acetic anhydride (0.6 mL) and catalytic amount of DMAP were added. The mixture was stirred at r.t. (room temperature) until the starting material disappeared by TLC (~ 2 h). The solvent was evaporated and the crude was redissolved in EtOAc. The ethyl acetate solution was washed with water, NaHCO₃ and brine. The evaporation of EtOAc gave the crude compound **(5)**. It was detritylated using 80% AcOH (10mL). After the evaporation of AcOH, the crude was triturated with CH₂Cl₂ / Ether to obtain pure **(6)**.

Dimer (8)

To a solution of **(3)** (1 mmol) in anhydrous CH₃CN (30 mL), **(6)** (1 mmol) was added. The reaction mixture was stirred under argon for 10-15 min. To this solution tetrazole (3 mmol) was added and stirred for 3-4 h. Then the solvent was evaporated. The obtained crude was triturated with 70:30 EtOAc / Ether and filtered. The filtrate was evaporated to afford the crude dimer. This was used in the next step without further purification. The crude dimer was dissolved in the mixture of THF/Pyridine/H₂O (8:2:0.2 mL) and iodine crystals (50-100 mg) were added portionwise until the iodine color persisted. Then excess iodine was neutralized by the addition of saturated solution of sodium thiosulfate. The solvent was evaporated and the crude was dissolved in EtOAc, washed with water, NaHCO₃ and brine. After evaporation of the solvent, the crude was stirred with 80% AcOH (20 mL) for 1 hour. AcOH was evaporated and the compound was purified by flash chromatography on silica gel using 10-15 % MeOH/ CHCl₃. The product obtained was treated with NH₄OH (100 mL) overnight. After evaporating the NH₄OH, it was purified on a DEAE cellulose ion exchange column using gradients of NH₄CO₃ buffer (0.02 to 0.2 M). The fractions were collected and lyophylized to give pure dimer **(8)**.

III.1.3. Phosphorothioate Dimers

This compound was prepared from the phosphodiester dimers in Scheme 3 by means of thiolation reaction. The procedure used is standard technique for the synthesis of backbone modified phosphorothioate oligonucleotides [4]. The thiolation reaction sequence is illustrated in Scheme 3. The phosphorothioate dimers have been proposed in addition to the normal phosphoroester dimers as they would have a reduced penchant for hydrolysis in plasma prior to entering the infected erythrocytes.
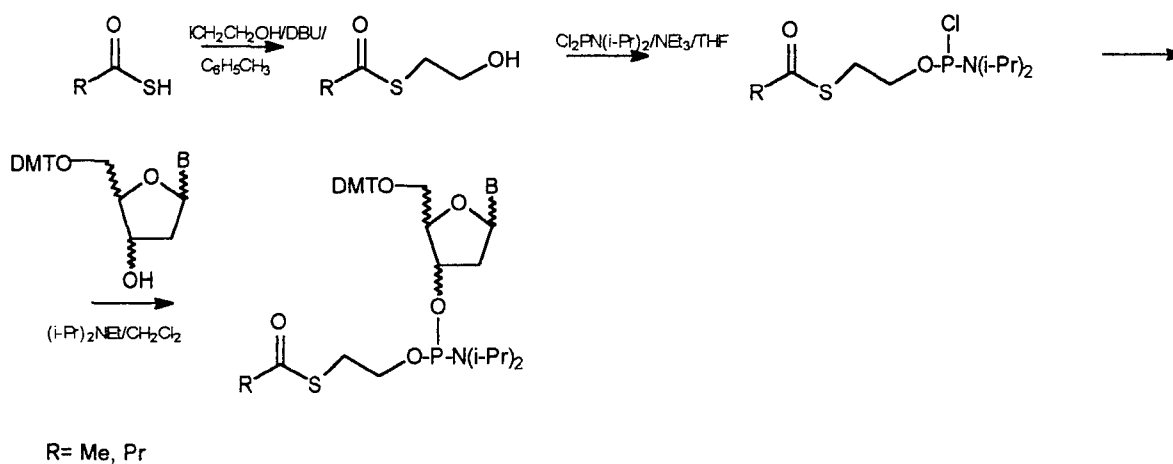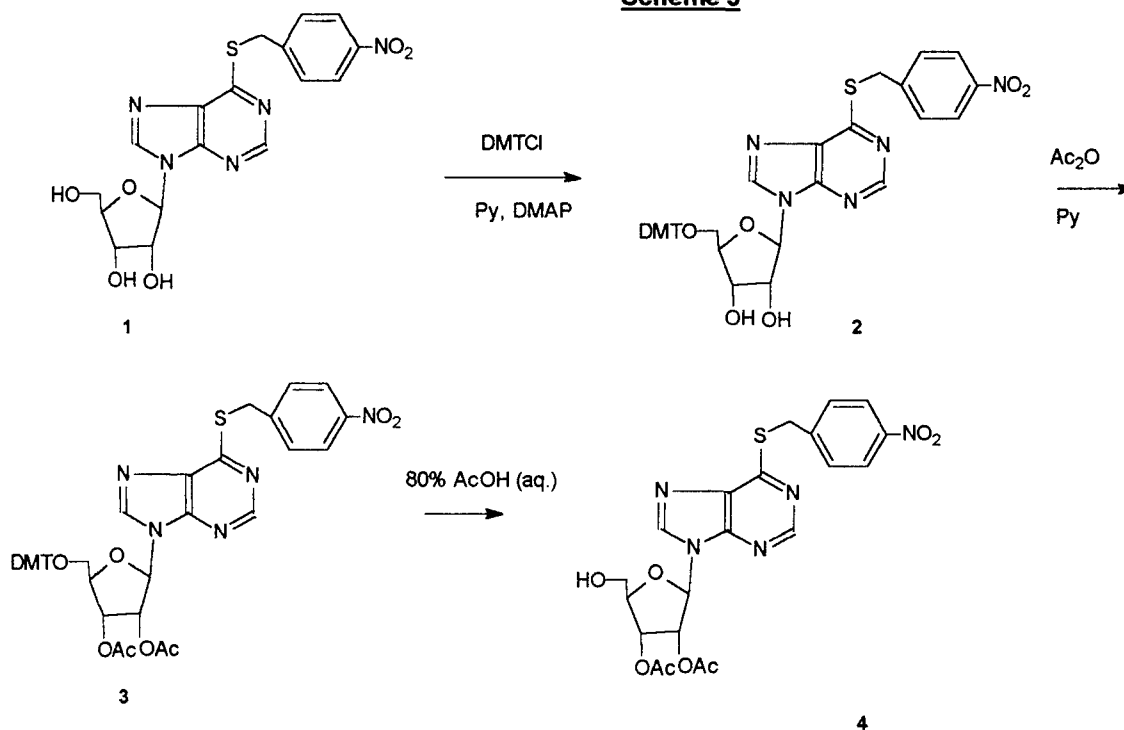
Scheme 3
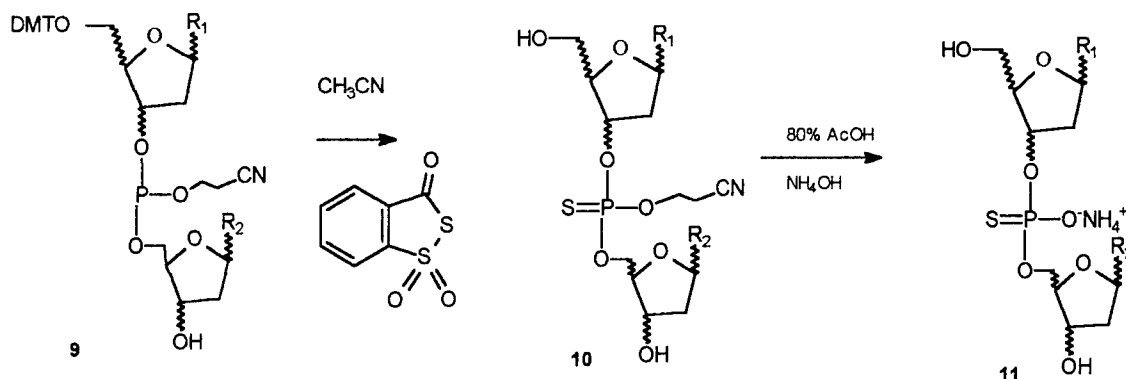
To a solution of **(3)** (1 mmol) in anhydrous CH_3CN (30 mL), **(6)** (1 mmol) was added. The mixture was stirred at r.t. under argon for 10-15 min. To this solution tetrazole (3 mmol) was added and stirring was continued for 3-4 h. A solution of 3H-1,2-benzoditiol-3-one 1,1-dioxide (4 eq.) in CH_3CN (10 mL) was added to the reaction mixture and the solution was stirred for 1 h. The solids were filtered off and the filtrate was evaporated. The residue was dissolved in EtOAc. After the workup the crude **(10)** was treated with 80% AcOH (25 mL) for 1 h. AcOH was evaporated and the compound was purified by flash chromatography on silica gel using 10-15 % MeOH/ $CHCl_3$. The product obtained was treated with NH_4OH (100 mL) overnight. After evaporating the NH_4OH, it was purified on a DEAE cellulose ion exchange column using gradients of NH_4CO_3 buffer (0.02 to 0.2 M). The fractions were collected and lyophylized to give pure dimer **(11)**.

III.1.4. SATE Derivatives

The bis-S-acethylthioethyl (SATE) compounds designed specifically for kinase bypass have been prepared essentially by application of the method of Imbach, *et al* [5]. The process is illustrated in Scheme 4.

Scheme 4

R= Me, Pr

III.1.5. Synthesis of nitrobenzylthioinosine (NBMPR) containing dimer

The dimeric derivatives of purine nucleoside transport inhibitor NMBPR have been prepared as shown in Scheme 5.

Scheme 5

DMTCl
Py, DMAP

EtN(iPr)₂/CH₂Cl₂

5 **6** **7**

4 + 7

CH₃CN
1-H-Tetrazole

1. I₂/THF/Lutidine
2. 80% AcOH

8

NH₄OH

OAc OAc **9 & 10**

11: B= Uracil
12: B= Thymine
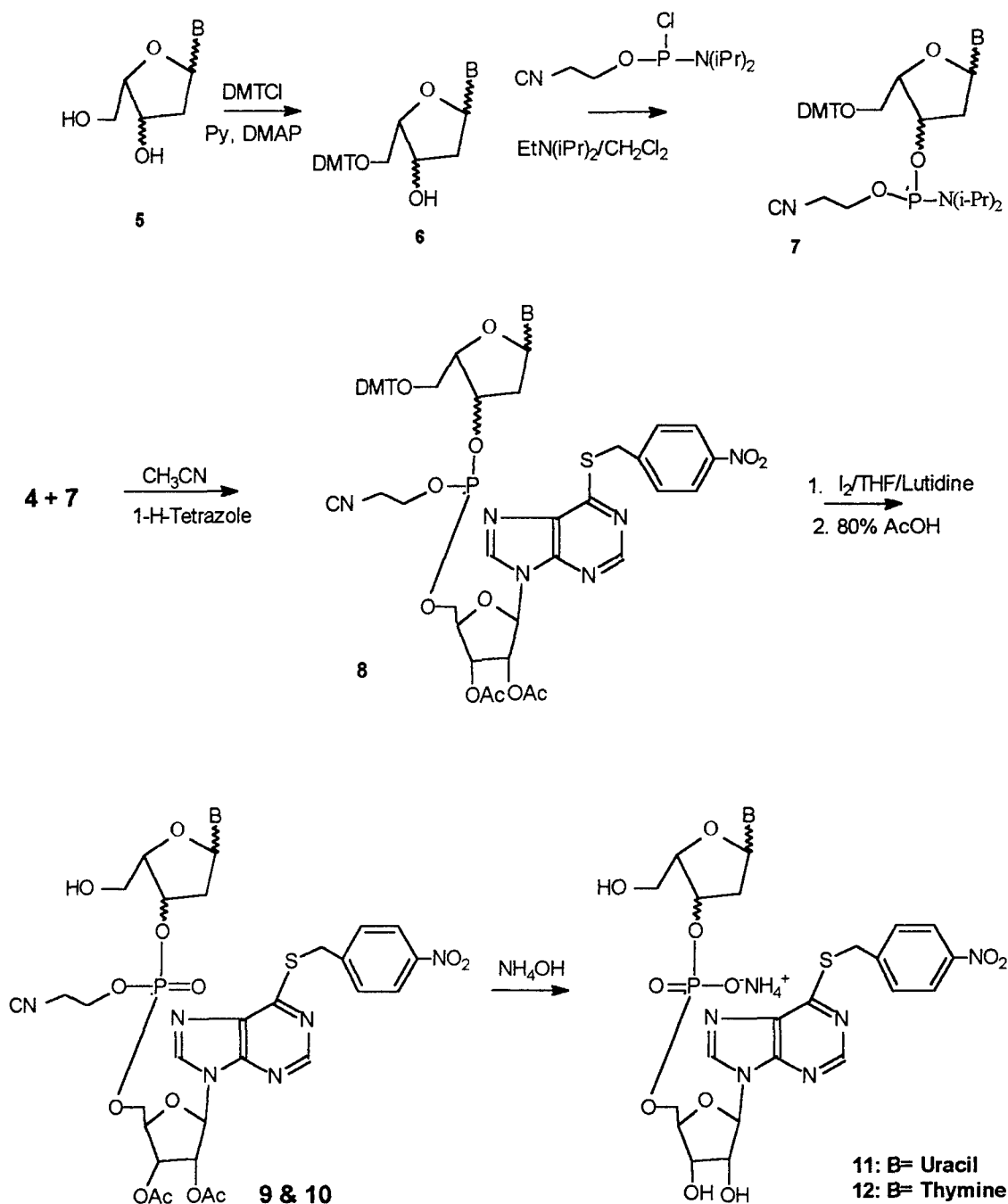
5'-O-(Dimethoxytrityl)-6-(4'-nitrobenzyl)-thio-9-β-D-ribofuranosylpurine (2)

To a stirred mixture of (1) (500 mg, 1.2 mmol), dimethylaminopyridine (30 mg, 0.24 mmol), pyridine (8 mL), dimethoxytrityl chloride (528 mg, 1.56 mmol) was added at 22°C. Reaction mixture was stirred at 22°C for 18 h. Then the reaction was quenched with methanol (0.5 mL) and the methanol and pyridine were evaporated *in vacuo*. The residue was taken up in ethyl acetate and water. Ethyl acetate was separated and aqueous layer was extracted with ethyl acetate. The combined ethyl acetate extract was washed with water, brine; dried (Na₂SO₄) and evaporated *in vacuo*. The crude compound was purified on flash column chromatography to afford pure (2) (780 mg, 90%).

5'-O-(Dimethoxytrityl)-2',3-diacetoxy-6-(4'-nitrobenzyl)-thio-9-β-D-ribofuranosylpurine (3):

Acetic anhydride (437 mg, 4.28 mmol) was added dropwise to a stirred mixture of **2** (770 mg, 1.07 mmol), dimethylaminopyridine (13 mg, 0.11 mmol), pyridine (8 mL) at 22°C. The reaction mixture was stirred at 22°C for 2 h. All the pyridine was evaporated *in vacuo* and the residue was taken in ethyl acetate and water. Ethyl acetate was separated and the aqueous layer was extracted with ethyl acetate. The combined ethyl acetate extract was washed with water, brine, dried (Na_2SO_4) and evaporated *in vacuo* to yield the diacetate **3** (862 mg, 100%).

3',4'-Diacetyl-6-(4'-nitrobenzyl)-thio-9-β-D-ribofuranosylpurine (4):

A mixture of **3** (780 mg, 1.07 mmol), 80% acetic acid (15 mL) was stirred at r.t. until the starting material 3 disappeared by TLC (60-90 min). All the acetic acid was evaporated *in vacuo* and the residue was purified by flash column chromatography to afford **4** (450 mg, 84%).

2'-Deoxy-5'-O-(Dimethoxytrityl)-β-L-uridine (6):

To a stirred mixture of 2'-Deoxy-β-L-uridine **5** (456 mg, 2 mmol), dimethyl-aminopyridine (50 mg, 0.4 mmol), pyridine (10 mL), dimethoxytrityl chloride (881 mg, 2.6 mmol) was added at 22°C. The reaction mixture was stirred at 22°C for 16 h. Then it was quenched with methanol (0.5 mL). The solvents were evaporated *in vacuo*. The residue was taken in ethyl acetate and water. Ethyl acetate was separated and the aqueous layer was extracted with ethyl acetate. The combined ethyl acetate extract was washed with water, brine, dried (Na_2SO_4) and evaporated in vacuo. The crude compound was purified by flash chromatography to afford pure **6** (850 mg, 80%).

2'-Deoxy-5'-O-(Dimethoxytrityl)-β-L-uridine-3'-N,N-diisopropylcyanoethyl phosphoramidite 7:

2'-Deoxy-5'-O-(Dimethoxytrityl)-β-L-uridine 6 (477 mg, 0.9 mmol) was dissolved in CH_2Cl_2 (20 mL). To this homogenous solution diisopropylethylamine (464 mg. 630 mL, 3.6 mmol) was added at 22°C. To the resulting red colored reaction mixture, 2'-cyanoethyl-N,N-diisopropylchlorophosphoramidite (276 mg, 260 mL, 1.17 mmol) was added dropwise at 22°C. The reaction mixture was stirred at 22°C until the starting material 6 disappeared by TLC (~ 30 min). The reaction was quenched with methanol (0.1 mL) and the solvent was evaporated *in vacuo*. The residue was partitioned between 80% ethyl acetate, triethylamine and water. The organic layer was separated, washed with saturated $NaHCO_3$ solution and brine. The solvent was evaporated and the residue was purified by flash chromatography (CH_2Cl_2 : EtOAc : Et_3N 45:45:10) to obtain a viscous liquid 7. This was dried *in vacuo* for 1 h and used in the next step immediately.

2'-Deoxy-5'-O-(Dimethoxytrityl)-3'-N,N-diisopropylcyanoethyl phosphoramidityl-[2',3'-diacetyl-6-(4'-nitrobenzyl)-thio-9-β-D-ribofuranosylpurinyl]-β-L-uridine (8):

Compounds 7 (657 mg, 0.9 mmol) and **4** (453 mg, 0.9 mmol) were dissolved in acetonitrile (30 mL). To this stirred solution sublimed tetrazole (189 mg, 2.7 mmol) was

added at 22°C. The reaction mixture was stirred at 22°C for 16 h. The acetonitrile was evaporated *in vacuo* and the residue was triturated with 70% EtOAc- ether mixture. The solid was filtered off and the filtrate was evaporated to give a foam **8**, which was used directly in the next step.

2'-Deoxy-5'-O-(Dimethoxytrityl)--3'-O-[2',3'-diacetyl-6-(4-nitrobenzyl)-thio-9-β-D-ribofuranosylpurinyl]-β-L-uridine cyanoethyl phosphonate ester (9):

Compound **8** was dissolved in THF:pyridine:H$_2$O (8:2:0.4 mL). To this stirred solution iodine crystals were added gradually until the iodine color persisted for ~ 10 min. Excess iodine was neutralized by the addition of few drops of saturated sodium thiosulfate. The solvent was evaporated and the residue was taken in ethyl acetate. The solution was washed with saturated NaHCO$_3$, brine, dried (Na$_2$SO$_4$) and evaporated *in vacuo* to obtain **9**. This compound was taken directly into next step without further purification.

2'-Deoxy-3'-O-[2',3'-diacetyl-6-(4-nitrobenzyl)-thio-9-β-D-ribofuranosylpurinyl]-β-L-uridine cyanoethyl phosphonate ester (10):

A mixture of **9** (840 mg) and 80% acetic acid (15 mL) was stirred at 22°C until the starting material disappeared by TLC (~ 60 min). The acetic acid was evaporated *in vacuo* and the crude compound was purified by flash chromatography to afford the pure compound 10 (270 mg).

2'-Deoxy-3'-O-[5'-O-6-(4'-nitrobenzyl)-thio-9-β-D-ribofuranosylpurinyl]-β-L-uridine phosphate, ammonium salt (11):

The protected dimer **10** (270 mg) was treated with ammonium hydroxide (30 mL, 30% ammonia solution) at 22°C and stirred at this temperature until the starting material is disappeared by TLC (~16 h). The solvent was evaporated *in vacuo* and the residue was purified on DEAE Sephadex A-25 cellulose ion exchange resin (eluted with NH$_4$CO$_3$ solution 0.02 M and 0.2 M). The fractions containing pure compound were collecteed and solvent was evaporated *in vacuo* and liophylized to obtain white compound **11** (65 mg).

2'-Deoxy-3'-O-[5'-O-6-(4'-nitrobenzyl)-thio-9-β-D-ribofuranosylpurinyl]-α-L-thymidine phosphate, ammonium salt (12):

The compound **12** was prepared the same as **11**, using 2'-deoxy-α-L-thymidine as a starting material.

III.1.6. Synthesis of Dimer with non-hydrolyzable "bridge".

Scheme 6

Where R = TBDMS

5+9 $\xrightarrow{\text{NaI, Na}_2\text{CO}_3, \text{CH}_3\text{CN}}$ **10** $\xrightarrow[\text{THF}]{n\text{-Bu}_4\text{NF}}$ **11**
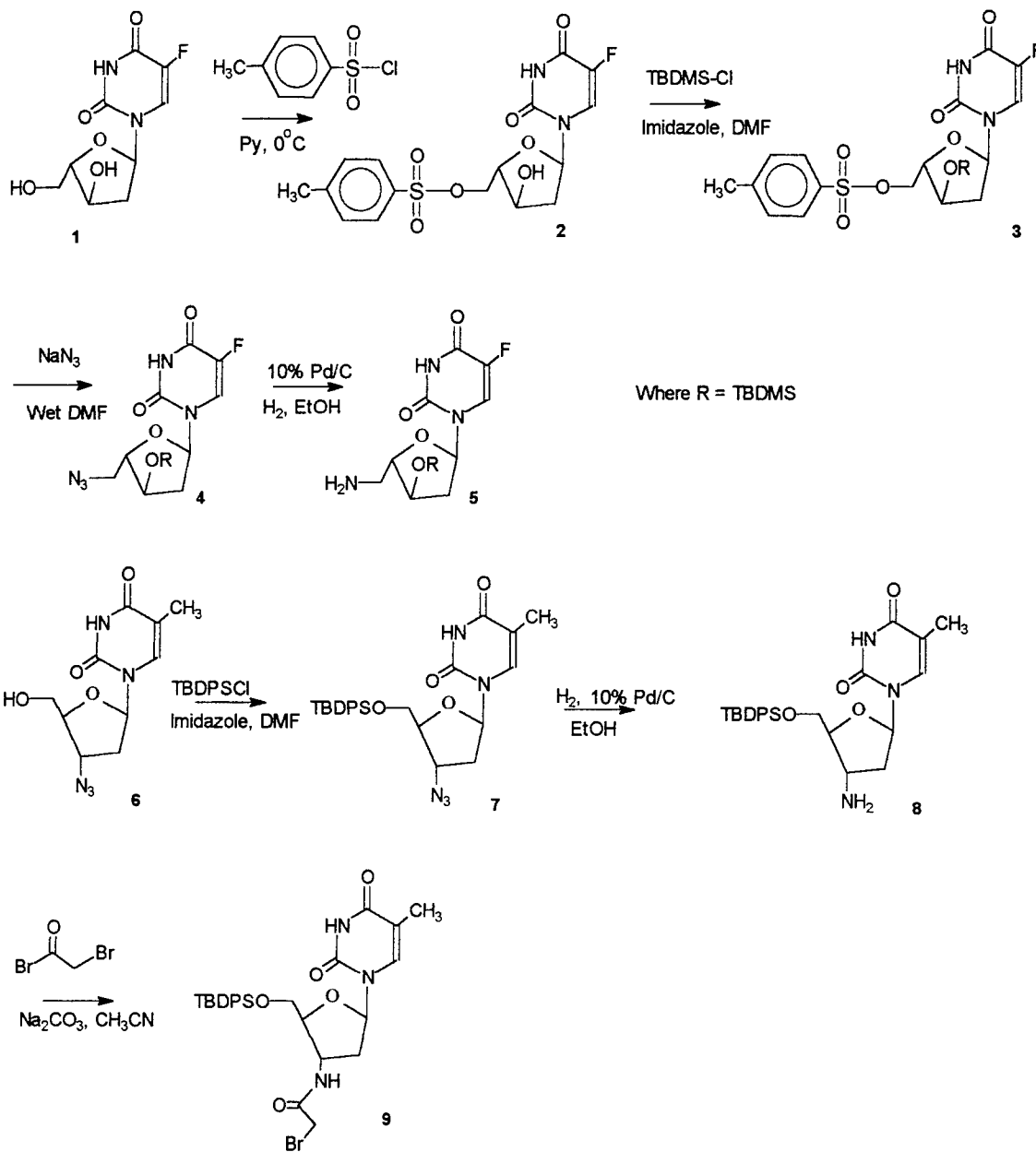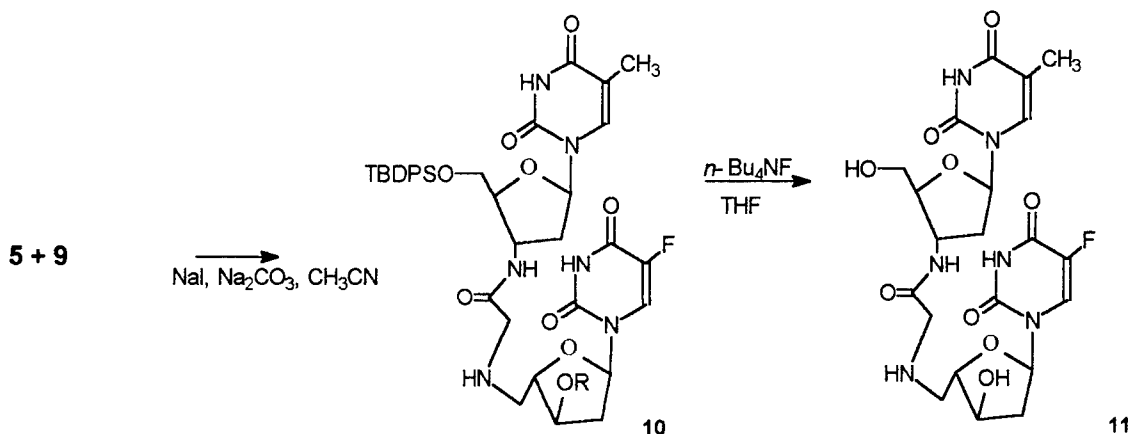
5'-O-*p*-Toluylsulfonyl-2-deoxy-α-L-thymidine (2) :

p-Toluenesulfonyl chloride (458 mg, 2.4 mmol) was added to a cold (0° C) solution of a-L-2-deoxythymidine **1** (492 mg, 2 mmol) in dry pyridine (10 ml). The reaction mixture was stirred at 0° for 20 h. The reaction mixture was treated with water (5 ml), and all the pyridine was evaporated *in vacuo*. The residue was taken in ethyl acetate and water. The ethyl acetate was separated and the aq. layer was extracted with ethyl acetate. The combined ethyl acetate extract was washed with water, brine and dried (Na₂SO₄) and evaporated *in vacuo* to obtain the tosylate **2** (760 mg, 95%) .

3'-O-*t*-Butyldimethylsilyl-5'-O-*p*-toluylsulfonyl-2'-deoxy-α-L-thymidine (3) :

t-Butyldimethylsilyl chloride (369 mg, 2.45 mmol) in dimethylformamide (2 ml) was added dropwise to the stirred mixture of the alcohol **2** (750 mg, 1.87 mmol), imidazole (318 mg, 4.67 mmol) in dimethylformamide (10 ml) at room temperature. The reaction mixture was stirred at room temperature for 14 h. Dimethylformamide was evaporated *in vacuo* and the residue was taken up in ethyl acetate (60 ml) and water (20 ml). The water layer was separated and extracted with ethyl acetate (3 x 30 ml). The combined ethyl acetate extracts were washed with water, brine, dried (Na₂SO₄) and evaporated *in vacuo* and the crude compound was purified by flash column chromatography to obtain the silylated compound **3** (800 mg, 83%).

5'-Azido-3'-O-*t*-butyldimethylsilyl-2',5'-dideoxy-α-L-thymidine (4) :

To a stirred mixture of the tosylate **3** (775 mg, 1.5 mmol) in dimethylformamide (15 ml) and water (0.5 ml) , sodium azide (490 mg , 7.5 mmol) was added at room temperature. The reaction mixture was stirred at 90-100°C (bath temperature) for 14 h. The reaction mixture was cooled to room temperature and dimethylformamide was carefully removed *in vacuo* at 25-30°C . The residue was diluted with water and extracted with ethyl acetate (3 x 50 ml) . The combined ethyl acetate extracts were washed with water , brine , dried (Na₂SO₄) and evaporated *in vacuo* to obtain the azide **4** (350 mg, 61%)

5'-Amino-3'-O-*t*-butyldimethylsilyl-2',5'-dideoxy-α-L-thymidine (5):

The azide **4** (350 mg, 0.91 mmol) in ethanol (25 ml), containing 10% Pd/C (100 mg) was hydrogenated at 50 *psi* for 14 h. The catalyst was filtered off through a small

pad of celite and the ethanol was evaporated *in vacuo* to obtain the amine **5** (250 mg, 76%).

3'-Azido-5'-O-*t*-butyldiphenylsilyl-2',3'-dideoxy-β-D-thymidine (7) :

To a mixture of azidothymidine **6** (700 mg, 2.62 mmol), imidazole (552 mg, 8.12 mmol) in dimethylformamide (10 ml), t-butyldiphenylsilyl chloride (936 mg, 890 µl, 3.41 mmol) was added dropwise at room temperature. The reaction mixture was stirred at room temperature under argon for 16 h. The dimethylformamide was evaporated *in vacuo* and the residue was taken up in ethyl acetate and water. The ethyl acetate layer was separated and the water layer was extracted further with ethyl acetate. The combined ethyl acetate extracts were washed with water, brine, dried (Na$_2$SO$_4$) and evaporated *in vacuo* to obtain the silylated compound **7** (1.190 g, 90%).

3'-Amino-5'-O-*t*-butyldiphenylsilyl-2',3'-dideoxy-β-D-thymidine (8) :

The azide **7** (1.32 g, 2.62 mmol) and 10% Pd/C (200 mg) in ethanol was hydrogenated at 60 psi in a Paar hydrogenation apparatus for 14 h. The catalyst was filtered off through a small pad of Celite and the filtrate was evaporated *in vacuo* to afford the amine **8** (1.130 g, 90%).

3'-(2-Bromoacetamido)-5'-O-*t*-butyldiphenylsilyl-2',3'-dideoxy-β-D-thymidine (9) :

To a stirred mixture of amine **8** (1.103 g, 2.3 mmol), sodium carbonate (244 mg, 2.3 mmol) in acetonitrile (35 ml), bromoacetyl bromide (464 mg, 2.3 mmol) in acetonitrile (5 ml) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 14h. All the acetonitrile was evaporated *in vacuo* and the residue was taken up in water (10 ml) and ethyl acetate (40 ml) . The water layer was separated and further extracted with ethyl acetate (2x 25 ml) .The combined ethyl acetate extracts were washed with water, brine, dried (Na$_2$SO$_4$) and evaporated *in vacuo* to obtain the amide **9** (1.243 g, 90%) .

5'-O-*t*-butyldiphenylsilyl-3'-acetamido-[3'-O-*t*-butyldimethylsilyl-2',5'-dideoxy-α-L-ribofuranosyluridinyl-5'-aminomethyl]-2',3'-dideoxy-β-D-thymidine (10) :

To a stirred mixture of the amine **5** (90 mg, 0.25 mmol), sodium carbonate (40 mg, 0.38 mmol), in acetonitrile (3 ml) , the bromide **9** (150 mg, 0.25 mmol) in acetonitrile (5 ml), sodium iodide (57 mg, 0.38 mmol) was added under argon. The reaction mixture was refluxed under argon for 14 h. The reaction mixture was cooled to 22^{0}C and the solid was filtered and washed with acetonitrile. The filtrate was evaporated *in vacuo* and the residue was taken in ethyl acetate (20 ml) and water (5 ml). The water layer was separated and further extracted with ethyl acetate. The combined ethyl acetate was washed with water, brine, dried (Na$_2$SO$_4$) and evaporated *in vacuo* . The crude compound was purified by flash column chromatography (eluted with 2% MeOH-CHCl$_3$) to afford the pure amide dimer **10** (135 mg, 62%) .

3'-Acetamido-[2',5'-dideoxy-α-L-ribofuranosyluridinyl-5'-aminomethyl]-2',3'-dideoxy-β-D-thymidine (11):

To a stirred solution of the silyl compound **10** (114 mg, 0.13 mmol) in tetrahydrofuran (5 ml), tetra-*n*-butylammonium fluoride (800 μL of 1M solution in tetrahydrofuran, 0.8 mmol), was added dropwise at 22^0C under argon. The reaction mixture was stirred under argon for 3h. All the tetrahydrofuran was evaporated *in vacuo* and the crude compound was purified by flash column chromatography (eluted with 15%-20% MeOH-CHCl₃) to afford the pure amide dimer **11** (63 mg, 94%)

III.1.7. Analysis of Test Compounds

Compounds have been characterized by melting points, proton, carbon and phosphorus NMR spectroscopy, thin layer chromatography, HPLC analysis and mass spectra.

III.2 BIOLOGICAL TESTING PROTOCOLS

All biological evaluations were performed in the laboratories of Dr. Annette Gero at the University of New South Wales.

III.2.1 *In vitro* Culture of the Malarial Parasite

Plasmodium falciparum , FCQ27, was maintained in culture using the techniques described by Trager & Jensen [6]. Cultures containing 2% hematocrit suspensions of parasitised human type O^+ erythrocytes in RPMI 1640 medium, supplemented with 25 mM HEPES-KOH, pH 7.2, 25 mM $NaHCO_3$ and 10% human type O^+ serum (v/v) are maintained in modular incubator chambers at 37°C in a gas mixture of 5% O_2, 5% CO_2 and 90% N_2. The isolate of *P.falciparum* used in these experiments was FCQ27, routinely maintained in synchronized or asynchronous *in vitro* cultures at low hematocrit.

III.2.2 *In Vitro* Toxicity against *P. falciparum*

The potential toxicity of nucleoside analogues against *P.falciparum* in culture was tested in microtitre plates over the range of drug concentrations for 24 hours. The procedures for monitoring parasite viability is well established [7] and is based on radiolabelled hypoxantine or isoleucine incorporation. The incorporation of [G-³H]hypoxantine into the nucleic acids of *P.falciparum* was used to assess the viability of the parasite *in vitro*. Microculture plates were prepared with each well containing 225 μl of a 2% hematocrit culture of asynchronous parasitised erythrocytes (1 % parasitised cells). Each plate, containing varying concentrations of the drug to be studied (up to 200 μM final concentration for initial screen), was incubated for 24 h at 37°C in a gas mixture of 5% O_2, 5% CO_2 and 90% N_2, at which point [G-³H] hypoxantine was added to each well and the incubation continued under identical conditions for a further 18-20 h. The control infected cells (ie. without drug), routinely reached a parasitemia of 6-8% before harvesting. Expediency was aided by 96-well plate counter using lactate dehydrogenase for the drug susceptibility assay [8,9]. This assay gave the identical results to the hypoxantine technique. In addition, for each experiment, microscopic counting of Giemsa stained thin slides was used as a control.

III.2.3 Transport and Metabolism in *P.falciparum* infected erythrocytes

The metabolism of the L-nucleoside conjugates was studied by HPLC analysis. The primary aim was to determine their ability to be catabolized by parasite purine salvage enzymes. Some effect on the purine metabolic pools was also observed.

For each HPLC determination 200 μL of packed cells of 80-90 % trophozoite infected cells were used. These were isolated from *in vitro* cultures by synchronization of the parasites in *in vitro* cultures using sterile D-sorbitol [10] followed by separation of the trophozoites from non infected erythrocytes by Percoll gradients as described previously [7]. Trophozoites were incubated at 37°C for 2 hours with each compound to be tested. Compounds were incubated with both whole infected cells as well whole and lyzed uninfected normal erythrocytes to determine:

a) entry to the cell (whether they were transported);

b) the metabolic effect within the cell (was the compound metabolized);

c) the capacity of broken or lyzed cells to catabolize the compound which may not be able to enter the unbroken cell (i.e. if the compound was transported into the cell, would it be metabolized to the active form).

Drug incubation was terminated by centrifugation through silicon oil using the method of Upston and Gero [11]. This procedure separated intact trophozoites from extracellular non-transported drug solution.

The metabolism of nucleosides with potential chemotherapeutic activity was assessed by the analysis of cytoplasmic samples by reverse phase ion-pair high performance liquid chromatography [12]. Nucleotides, nucleosides and bases were separated by this HPLC method.

IV. RESULTS AND DISCUSSION

The transport and metabolism of purine nucleosides differ considerably between the normal human erythrocyte and human erythrocytes which have been infected with *Plasmodium falciparum*. The malaria parasite is unable to synthesize purines *de novo* and relies on salvage pathways to obtain preformed purines it requires for growth and division [13]. Normal human erythrocytes do not contain significant levels of pyrimidine nucleotides [14], and the parasite is unable to obtain pyrimidine bases or nucleosides by salvage pathways and has to rely on *de novo* synthesis [13]. These modifications to the metabolic pathways of the infected erythrocytes, along with modifications of their transport system, represent significant variations from normal erythrocytes and may present an opportunity for the use of selectively toxic compounds against the parasites.

Nucleosides have attracted researchers as potential therapeutic agents. Naturally occurring nucleosides are usually in the β-D configuration. Therefore most of nucleoside analogues designed for the treatment of cancer, viral and parasitic diseases have been synthesized in this stereochemical configuration. Recent discoveries in our laboratories, at the University of Georgia , the University of Iowa and at Yale University, as well as at universities in France and Italy, have confirmed that most L-nucleosides exhibit low toxicity because normal cells do not utilize them for building RNA or DNA and don't

metabolize them. The discovery that 3TC (an approved treatment for HIV) was more potent and less toxic in its L-configuration than its enantiomer strongly supports the hypothesis that L-nucleosides and their conjugates could have therapeutic potential against invasive diseases such as cancer, viruses, and parasites [15,16,17].

Recently, Dr. Gero and her coworkers discovered that the nonphysiological β-L-adenosine can be selectively transported into a erythrocyte infected with *P. falciparum* [18]. Normal erythrocytes and other cell types are completely impermeable to this compound.

During the Phase I study, we used this unique ability of the non-natural nucleoside analogues for selective transport to the malaria infected cell to create a novel synthetic L-nucleoside based class of non-toxic antimalarial agents. Our working hypothesis was based on the design and biological evaluation of novel chemical entities which would consist of both 5-fluorodeoxyuridine (FUdR), a known inhibitor of thymidylate synthase, and an L-nucleoside or its derivatives. The number of "dimers" consisting of α- or β-L isomeric modification of physiological nucleosides or their derivatives was conjugated with FUdR by phosphate or pro-phosphate linkage. Along with anticancer activity, FUdR has a potential as an antimalarial agent [19]. Unfortunately, FUdR's toxicity limits its use. In theory, combining FUdR with an L-nucleoside unit would result in an entity that could selectively transport an active component to infected cells while having no effect on normal cells.

IV.1 RESULTS

During this Phase I research a total of 42 L-nucleoside analogs were screened in an *in vitro* assay against *P. falciparum*. From this forty two compounds, 31 were available for screening from Lipitek International's library and 11 were specifically synthesized for the purpose of this project. The detailed synthesis of 11 L-nucleoside conjugates is described in the Methods and Procedures. They were prepared in 100 mg scale and were fully characterized by analytical methods (NMR, HPLC, mass spectra, TLC). The forty two compounds tested were representative of L-nucleoside monomers or 4 different types of L-nucleosides conjugates. The conjugates tested were: a) dinucleoside phosphates, b) dinucleoside phosphorothioates, c) SATE derivatives of L-nucleosides, and d) L-nucleoside conjugates of nitrobenzylthionosine (NBMPR). It should be emphasized that even more diversification resulted from utilizing characteristic to nucleosides 3' to 5' versus 5' to 3' phosphodiester linkages as well as variations of purines and pyrimidines in both parts of the dimers.

The biological screen involved evaluation of the compounds against the protozoan *P. falciparum* in *in vitro* culture. The range of drug concentrations was tested independently by two assays. One, radiolabeled hypoxanthine incorporation into the nucleic acid of *P. falciparum,* and the other, more expedient assay, a 96-well plate susceptibility assay using lactate dehydrogenase. Both assays gave identical results. In addition, microscopic counting of Giemsa stained thin slides was used as a control. The results of the biological assays are presented in Table 1. Examples of experimental curves are attached as Appendix 2. The biological tests were done at several concentrations. The

highest concentration was 200 μM , the compounds were considered active at concentrations less than 40μM.

Fifteen dimers from the Lipitek's library were submitted for the U.S. Army Antimalarial Test Program (laboratory of Dr. Kyle) for bioevaluation. The compounds were tested for *in vitro* activity against two *P.falciparum* strains: D6 (non-chloroquine resistant) and W2 (chloroquine resistant). Results are attached as Appendix 3.

Ten tested compounds were selected for preliminary evaluation of metabolism and transport by HPLC. The results are summarized in Table 8. Representative examples are attached as Appendix 4.

IV.2. DISCUSSIONS

IV.2.1. *In vitro* activity

A careful analysis of the data presented in table 1 (below) indicates that nine (9) analogs from 42 screened had IC_{50} less than 40μM (for the structure of the tested compounds see appendix 1). The most active representative of dinucleoside phosphates were L-101, L-103, L-110, L-111, L-113, L-133 and L-138.

Table 1. Results of the *in vitro* testing.

N	CODE	COMPOUND	IC_{50}, μM
1.	L-101	[β-D]$_2$-FUdR	15
2.	L-103	[α-L,β-D]-FUdR	20
3.	L-103 thio	[α-L,β-D]-FUdR, S=P-O⁻	>200
4.	L-109	[β-L]$_2$-FUdR	>200
5.	L-110	[β-L, β-D]-FUdR	20
6.	L-111	α-L-dC, β-D-FUdR	38
7.	L-113	β-L-dC, β-D-FUdR	17
8.	L-117	β-L-dU, β-D-FUdR	35
9.	L-117 thio	β-L-dU, β-D-FUdR S=P-O⁻	>200
10.	L-125	α-L-dA, β-D-FUdR	60
11.	L-128	[β-D, β-D-FUdR] S=P-O⁻	1.5
12.	L-133	β-L-dG, β-D-FUdR	14
13.	L-138	β-L-dA, β-D-FUdR	5
14.	L-138-thio	β-L-dA, β-D-FUdR, S=P-O⁻	100
15.	L-144	β-D-FUdR, β-L-A	140
16.	L-145	β-L-dU, NBMPR	>200
17.	L-146	α-L-dT, NBMPR	>200
18.	L-147	NBMPR, MP	Solubility problem
19.	NBMPR	NBMPR	100
20.	GCI 1007	α-L-erythrofuranosyl-5-fluorouracil	>200

Table 1 (continued)

N	CODE	COMPOUND	IC$_{50}$, μM
21.	GCI 1018	α-L-arabinofuranosyl adenine	>200
22.	GCI 1027	α-L-FUdR	>200
23.	GCI 1030	β-L-G	>200
24.	GCI 1032	β-L-A	>200
25.	GCI 1033	β-L-I	>200
26.	GCI 1034	β-L-mercapto-G	>200
27.	GCI 1036	β-L-dA	>200
28.	GCI 1037	β-L-dI	>200
29.	GCI 1066	α-L-A	>200
30.	GCI 1069	α-L-dA	>200
31.	GCI 1070	β-L-dG	>200
32.	GCI 1077	β-L-ddA	>200
33.	GCI 1079	α-L-ddA	>200
34.	GCI 1085	N^6-methyl-β-L-A	>200
35.	GCI 1076	6-thio-β-L-purine	>200
36.	B01	β-D-FUdR 3'SATE	100
37.	B02	β-D-FUdR 5'SATE	60
38.	B03	β-D-FUdR 3'-5'SATE	6
39.	B04	β-L-FUdR 5' SATE	200
40.	B05	β-L-FUdR 3'-5'SATE	Solubility problem
41.	B06	α-L-FUdR 5'SATE	150
42.	B07	α-L-FUdR 3'-5'SATE	Solubility problem

The 14 L-nucleoside monomers in α- and β- forms and even α-L-FUdR showed no activity against *P. falciparum*. Because of that, further research on monomers was halted (see Table 1).

The dimer containing only the "non-natural" isomeric form of nucleoside (L-109) didn't exhibit any activity.

Careful analysis of the data in Table 1 indicates that β-D-isomer of FUdR is the active component of the dimer molecules. The position of the active component in the dimer is important. The β-D-FUdR needs to be connected to the 3'-OH end of the L-nucleoside through a phosphodiester linkage to its 5'-OH. Compounds which are linked through 3'-OH of FUdR are much less active (see Table 2). This indicates that the substitution pattern of β-D-FUdR is critical for the activity of the dimers and most probably the mechanism involves thymidylate synthase inhibition. It is well known that TS inhibitors of FUdR have very rigid structural requirements and do not allow for any substitution at the 3' end.

Table 2. The activity of the L-nucleoside containing dimers *versus* position of FUdR linkage

5'		3'	
Compound	IC_{50}, μM	Compound	IC_{50}, μM
L-101 ([β-D]₂-FUdR)	15	L-144 (β-D-FUdR, β-L-A)	140
L-103 (α-L-FUdR, β-D-FUdR)	20		
L-110 (β-L-FUdR, β-D-FUdR)	20		
L-111 (α-L-dC, β-D-FUdR)	38		
L-113 (β-L-dC, β-D-FUdR)	17		
L-125 (α-L-dA, β-D-FUdR)	60		
L-133 (β-L-dC, β-D-FUdR)	14		
L-138 (β-L-dA, β-D-FUdR)	5		

In the case of purine nucleoside, the attachment of the α-L nucleoside to β-D-FUdR monomer reduces the dimer activity in comparison with dimers containing the β-L unit (see Table 3 , L-125 & L-138). In the case of pyrimidine nucleosides there is no obvious difference in the activity (L-103 & L-110, L-111 & L-113, Table 3).

Table 3. The activity of the dimers *versus* chemical configuration of the L-nucleoside

α-L		β-L	
Compound	IC_{50}, μM	Compound	IC_{50}, μM
L-103 (α-L-FUdR)	20	L-110 (β-L-FUdR)	20
L-111 (α-L-dC)	38	L-113 (β-L-dC)	17
L-125 (α-L-dA)	60	L-138 (β-L-dA)	5

The different activity of the dimers is dependent on the structure of the second nucleoside.

Plausible pathways for metabolic activation and/or mode of action of the dimer molecules tested could be:

(1)   Dimer may act as a new chemical entity without hydrolysis of the phosphate or pro-phosphate bond between the two monomeric units;

(2)   Hydrolysis to L-nucleoside and FUdR nucleotide may occur; in which case the dimer is a prodrug. The L-nucleoside is used for protection and to increase the bioavailability of β-D-FUdR monophosphate.

It is also important to note that hydrolysis can take place intracellularly as well as outside the cell.

In the last decade, monumental efforts have been directed toward the synthesis of oligonucleotide analogs with altered phosphodiester linkage. The goal was to improve the stability of duplex and triplex formation, to improve the cellular uptake and to decrease the rate of degradation of oligonucleotides by endo and exo nucleases which cleave the phosphodiester linkage. We selected one such chemical modification for our study. As a consequence, several dimers with phosphorothioate linkage between two nucleosides were

synthesized and tested. The phosphorothioate comprises a sulfur-for-oxygen substitution at phosphorus of the phosphodiester linkage (for the structure of the corresponding dimers see Appendix 1). It has been shown [20] that the S homologues are more resistant to cellular nucleases and are readily taken up by cells. Several oligonucleotides of this type are currently in clinical studies (ISIS Pharmaceuticals and others).

Table 4 . The activity of the dimers *versus* nature of the linkage between two nucleosides analogs

Phosphate "bridge" O=P-O⁻	IC$_{50}$, μM	Phosphorothioate "bridge" S=P-O⁻	IC$_{50}$, μM
L-101 ([β-D]₂-FUdR)	15	L-128 ([β-D]₂-FUdR)	1.5
L-103 ([α-L,β-D]-FUdR	20	L-103 ([α-L,β-D]-FUdR	>200
L-117 (β-L-dU, β-D-FUdR)	35	L-117 (β-L-dU, β-D-FUdR)	>200
L-138 (β-L-dA, β-D-FUdR)	5	L-138 (β-L-dA, β-D-FUdR)	100

The replacement of the phosphate linkage by the phosphorothioate bond in the dimer, containing two β-D-FUdR units, increases the activity of the compounds by a factor of 10 (see Table 4, data for L-101 & L-128). The only active phosphorothioate analog appears to be compound L-128. The activity of L-128 is greater in comparison with all possible products of hydrolysis (see Table 5). Moreover, L-128 was the most active compound tested.

Table 5. The activity of β-D-FUdR and some possible products of its metabolism

COMPOUND	IC$_{50}$, μM
β-D-FUdR	34
β-D-5'-FUdRmP	50
5FUracil	6

The introduction of the phosphorothioate bond into molecules of dimers containing "non-natural" nucleoside isomer was not successful: the activity of the compounds was reduced dramatically (see Table 4, data for L-103, L-117 & L-138). As was discussed before, one of the possible mechanisms of dimer action is the participation in the metabolic pathways of the whole non-hydrolyzed molecule. In this case the increasing of the dimer stability by the introduction of the phosphorothioate linkage results in the increasing of the activity of L-101. For the dimers containing the "non-natural" isomeric modification of the nucleoside the metabolism of whole non-hydrolyzed molecule is probably impossible.

To confirm mechanism and improve the efficacy, design, synthesis, and bioevaluation of a number non-cleavable dimers will be proposed in the Phase II application.

It is well established that most of the nucleoside analogs are dependent on kinase-mediated activation to generate the bioactive nucleotide and ultimately, the nucleoside triphosphate [21]. Activation takes place in the cytosol after nucleoside uptake and involves three successive viral and/or cellular kinases, the first one being highly specific

[22]. One possibility to improve the efficiency of the nucleoside analog as a therapeutic agent could be to bypass the phosphorylation step. Unfortunately, nucleoside monophosphates themselves, due to their polar nature, are not able to cross the cell membrane efficiently [23]. Hence the idea of temporarily masking or reducing the phosphate negative charges with neutral substituents, thereby forming more lipophilic derivatives which would be expected to revert back to the nucleoside mono-phosphate once inside the cell.

One of the possible structural modification for the kinase bypass is the use of the bis-*S*-acetylthioethyl (SATE) derivatives pioneered by J.-L. Imbach [5,24]. We synthesized several SATE derivatives of FUdR isomers and tested their *in vitro* activity against *P.falciparum*. The obtained results are listed in Table 6.

Table 6. The activity of the SATE derivatives of FUdR

Compound	ID_{50}, µM
β-D-FUdR	34
β-D-FUdR monophosphate	50
B01 (β-D-FUdR 3' SATE)	100
B02 (β-D-FUdR 5' SATE)	60
B03 (β-D-FUdR 3',5' SATE)	6
β-L-FUdR	>200
β-L-FUdR monophosphate	N/A
B04 (β-L-FUdR 5' SATE)	200
B05 (β-L-FUdR 3',5' SATE)	Solubility problem
α-L-FUdR	>200
α-L-FUdR monophosphate	N/A
B06 (α-L-FUdR 5' SATE)	150
B07 (α-L-FUdR 3',5' SATE)	Solubility problem

It should be emphasized that all of the SATE derivatization was performed on monomers of FUdR varying the conformation. Thus derivatives of α- and β D and L-FUdR were prepared. Three types of SATE analogs were produced, a) decorated at 5' of the nucleoside, b) decorated at 3' of the nucleoside, and c) decorated at both 3' and 5' of the nucleoside resulting in disubstitution. Most of the SATE derivatives synthesized were inactive in the screening. Only B03, which is a disubstituted SATE derivative of β-D-FUdR, showed activity warranting further investigation.

Three derivatives of NBMPR were synthesized. Unfortunately, none of them demonstrated any activity in the assays.

Fifteen L-nucleoside dimers (L-101, L-103, L-103A, L-107, L-110, L-111, L-112, L-114, L-117, L-120, L-122, L-124, L-125, L-133 & L-138) from Lipitek's library were submitted for *in vitro* screen to the U.S.Army Antimalarial Test Program (for the structures of the compounds see Appendix 1). The compounds have been tested for their activity against two *P. falciparum* strains: D6 (chloroquin non-resistant) and W2 (chloroquin resistant). The results are attached as Appendix 3. Seven (7) of the tested compounds exibited activities below 40 µM against both strains of *P.falciparum*. The

most active dimers were L-101, L-110, L-112, L-117, L-133 & L-138. This results are complete agreement with the results obtained at the Dr. Gero's labs.

IV.2.2. The transport and metabolism study

It has been established that transport and uptake in parasite invaded cells is different from that of normal blood erythrocytes [25]. Invasion by the malaria parasite compromises the cell membrane, allowing penetration of unnatural substances of various size and shape. Whereas normal cells are very selective in uptake. It was shown that L-nucleosides and their derivatives easily penetrate invaded cells, while they have a very slow rate of uptake into normal cells, if they enter at all. In order to obtain preliminary data on transport , uptake and metabolism, in this Phase I study the HPLC method was used to analyze the following 10 Lipitek compounds: L-101, L-103, L-109, L-111, L-117, L-133, L-138, GCI 1007, GCI 1027, GCI 1069.

HPLC retention times for standard compounds purchased from Sigma is presented in Table 7.

Table 7. HPLC Retention Time for the Standard Compounds

Compound	R.t., min	Compound	R.t., min
Nucleobase		**Nucleotide**	
Adenine	4.93	5'AMP	16.56
Guanine	5.38	3'AMP	19.46
Uracil	4.37	5'ADP	23.89
Hypoxanthine	5.59	5'-ATP	30.09
Nucleoside		5'GMP	15.24
Adenosine	12.47	5'-GDP	23.13
Guanosine	9.96	5'GTP	29.01
Inosine	8.88	5'-UMP	14.85
Thymidine	12.36	FUdRMP	16.40
2'-Deoxyadenosine	12.29		
2'-Deoxyguanosine	11.03		
2'-Deoxyuridine	7.41		
2'-Deoxycytidine	8.25		
FUdR	10.21		

These compounds were chosen for the identification of possible metabolites. IN this experiment the compounds were incubated with both whole infected cells, and with whole and lysed uninfected cells, followed by the separation of unreacted compound and HPLC analysis.

The results are presented in Table 8:
Column 1 shows the retention times of the original compound (not incubated with any cells).

The results are presented in Table 8:
Column 1 shows the retention times of the original compound (not incubated with any cells).
Column 2 shows the retention times of the original compound remaining after incubation with whole parasite infected cell.
Column 3 shows the metabolic products i.e. new peaks due to conversion of the original compound or alteration in the natural purine or pyrimidine profile of the infected cell.

Table 8. **HPLC Retention Time of Lipitek's Compounds (Trophozoite Incubations)**

Compound	Neat Injection	Unmetabolized Peak	Metabolic Product
L-138	18.17, 18.89	18.26	16.93, 15.73
L-133	16.72	17.03	15.65
L-101	19.05	19.01	10.62
L-103	18.31	18.25	-
L-117	17.41	17.61	-
L-111	15.69	15.69	-
L-109	16.36	-	-
GCI 1027	10.21	10.61	-
GCI 1007	12.61	12.42	-
GCI 1069	12.29	-	9.65

All nucleosides monophosphate dimers containing β-D-FUdR unit in combination with any L-nucleosides (L-101, L-103, L-111, L-117, L-133, and L-138) as well as tested L-nucleoside monomer analogs (GCI 1007, GCI 1027 & GCI 1069) entered the infected cells. All these compounds were toxic against P. falciparum. The L-109, combination of two L-dimers, could not enter the infected cell, and was also not toxic. .

Compounds L-101, L-133 & L-138 appear to be metabolized by the infected cells, each producing at least one new peak (see Appendix 4 & Table 8). It is possible that L-138 and L-133 may be cleaved to a nucleotide and nucleoside. Further investigation using radiolabeled substrates will be proposed for Phase II investigation.

None of the above 10 compounds were found to enter normal erythrocyte. Metabolism of any of the above compounds didn't occur in lysates of human erythrocytes or lymphocytes (see Appendix 4 for the examples). So even if the compounds were able to get into the normal cells, the normal cells can't metabolize them into active ingredients. This underscores again the low toxicity and the selectivity of Lipitek's L-nucleoside conjugates.

The complexity of the HPLC data for the trophozoite analysis is a problem. Based on the crude HPLC data, we are not able to state definitively whether the dimers are hydrolyzed by the malarial cells, and if so, what the products are. Enormous amount of cells were needed for the analysis of each compound due to the low sensitivity of HPLC analysis. The difficulty is knowing whether the "new" or different peaks (compared to the trophozoite control without the drug), result from a metabolite appearing, or an increase in a metabolite due to the inhibition of a metabolic pathway by the dimer.

One possible way to obtain data which would be easy to analyze is to radiolabel a few of the interesting compounds. This also makes possible transport assays which would yield clean and definite results with minimal amounts of material. This is another subject of investigation in Phase II.

V. CONCLUSION

During the Phase I research over 40 compounds were tested for *in vitro* activity against *P. falciparum*. For 10 of the compounds, the transport to infected and normal erythrocytes has been studied. For several compounds, the products of the metabolism inside the infected cell have been analyzed using HPLC techniques.

Most of the dinucleoside phosphate derivatives containing L-nucleoside and β-D-FUdR were active. Compound L-138, a dimer of β-L-dA and β-D-FUdR yielded the best efficacy with an $IC_{50}=5$ μM. One phosphothioate, L-128, showed even better activity at $IC_{50}=1.5$ μM. The disubstituted SATE derivative of β-D-FUdR showed $IC_{50}=6$ μM. It should be emphasized that an independent screening effort by the U.S. Army Antimalarial Test Program confirmed the obtained results.

L-nucleosides and their conjugates consistently demonstrate low toxicity profiles and our recent *in vivo* testing showed MTD's over 500 mg/kg in anticancer assays. This makes the results of this study even more exciting, because of the high selectivity and low toxicity of the compounds tested. In our view, the efficacy is secondary to selectivity and toxicity.

The encouraging preliminary results obtained in this Phase I research warrant a full scale, detailed Phase II research effort, which will be direct toward
   a) design of new conjugates, with even better therapeutic profiles,
   b) establishment quantitative structure/activity relationship of synthesized
      substances based on biological evaluation,
   c) determination of mechanism of action,
   d) study transport, uptake and metabolism using radiolabeled models,
   e) conduction *in vivo* studies with selected drug leads,
   f) nomination drug candidate for development.

VI. LITERATURE

1.   R.H. Barker, Jr., V. Metelev, E. Rapaport, P. Zamecnik, Proc. Natl. Acad. Sci. USA. **93** 514-518 (1996).

2.   H. Ginsburg, Biochem. Pharmacol. **48**, 1847-1856 (1994).

3.   A.M. Gero, W.J. O'Sillivan, Blood Cells. **16**, 457-484 (1990).

4.   G. Beaton, D. Pellinqer, W.S. Marshall & M. H. Caruthers, In: Oliqonucleotides and Analogies. A Practical Approach, F. Eckstein, Ed; IRL Press, Oxford. 109-136 (1991).

5.   I. Lefebvre, C. Perigaud, A. Pompon, A.M. Auberth, J.L. Grardet, A. Kirn, G. Gosselin and J.L. Imbach, J. Med. Chem. **38**, 3941-3950 (1995).

6.   W. Traqer and J.B. Jensen, Science. **193** 673-675 (1976).

7.   A.M. Gero, H.V. Scott, W. J. O'Sullivan and R.I. Christopherson, Mol. Biochem. Parasitol. **34**, 87-89 (1989).

8.   L. K. Basco, F. Marguet, M.T. Makler and J. Lebbras, Exp. Parasitol. **80**, 260-271 (1995).

9.   M.T. Makler and D.J. Hinrichs, Am. J. Trop. Med. Hgy. **48**, 205-210 (1993).

10.   L. Lambros and J.P. Vanderberg, Parasitol. **65**, 418-420 (1979).

11.   J.M. Upston and A.M. Gero, Biochem. Biophys. Acta. **1236**, 249-258 (1995).

12.   R.S. Toguzov, Y.V. Tikhonov, A.M. Pimenov, V. Prokudin, Journal of Chromatography, I. Biomedical Appl. **434**, 447-453 (1988).

13.   L.W. Scheibel & T.W. Sherman, In: Malaria: Principles and practice of Malariology (W.H.Wernsdorfer & I. McGregor, Eds.) V.1, 234-242 (1988).

14.   E. Szabodos & R.I. Christopherson, Biochem. Educ. **19**, 90-94 (1991).

15.   C.N. Chang, S.L. Doong et al. J. Biol. Chem. **267**, 13939-13942 (1992).

16. H. Soudens, X.J. Yao, Q. Gao, B. Belleau, J.L. Kraus, N. Nguyen-Ba, B. Spira, M.A. Wainberg, Antimicrob.Agents Chemother. **35**, 1386-1390 (1991).

17. T.S. Lin, M.-Z. Luo, M.-C. Liu, S.B. Pai, G.E. Dutschman & Y.-C. Chen, J.Med.Chem. **37**, 798-803 (1994).

18. A.M. Gero & J.M. Upston, In: Pyrine & Pyrimidine Methabolism in Man VIII (A. Sahota & M. Taylor, Eds) Plenum Press, NY, 495-498 (1995).

19. S.A. Queen, D.L. Vander Jagt & P. Reyer, Antimicrobial Agents & Chemotherapy. **34,** 1393-1398 (1990).

20. M. Matsukura, K. Shinozuka, G. Zon, H. Mitsuya, M. Reitz, J.S. Cohen, L.M. Neckers, Proc. Natl. Acad. Sci. USA. **84**, 7706 (1987).

21. C. Periqaud, G. Gosselin, J.L. Imbach, Nucleosides Nucleotides. **11**, 903 (1992).

22. M.C. Starnes, Y.C. Cheng, J. Biol. Chem. **262**, 988 (1987)

23. K.C. Leibman, C.J. Heidelberg, J. Biol. Chem. **216**, 823 91995).

24. C. Perigaud, G. Gosselin, I. Lefebvre, J.L. Girardet, S. Benzaria, I. Barber and J.L. Imbach, Bio Org. Med. Chem. Lett. **3,** 2521-2526 (1993).

25. A.M. Gero & A.M. Wood, In: Pyrine & Pyrimidine Methabolism in Man VII, Part A. (R.A.Harkness et al., Eds) Plenum Press, NY, 169-172 (1991).

VII. APPENDIX 1
Structures of the Tested Compounds
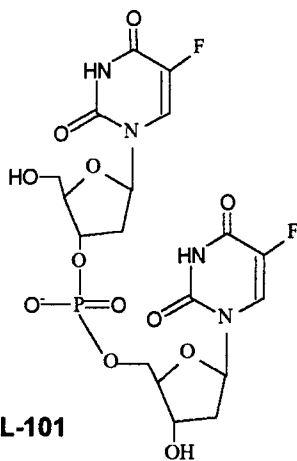
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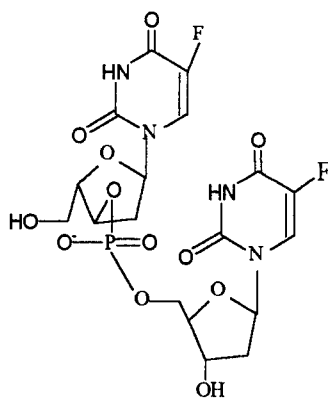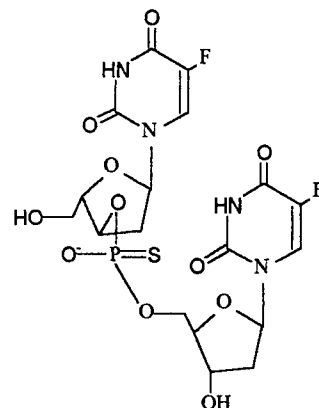
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L-103 thio
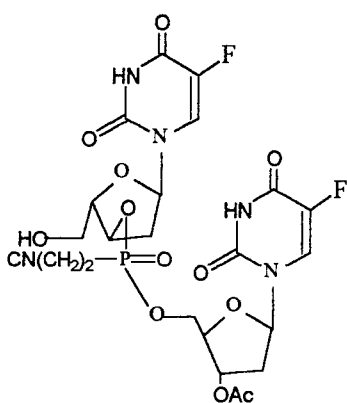
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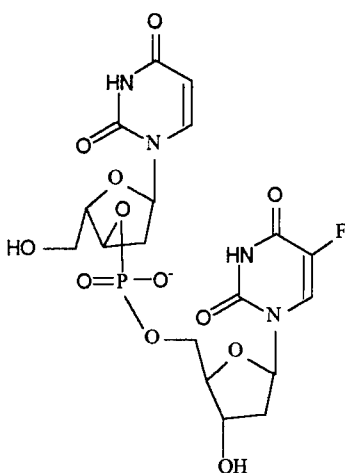
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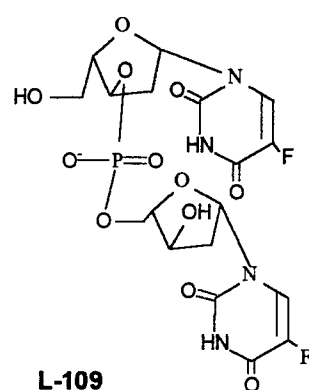
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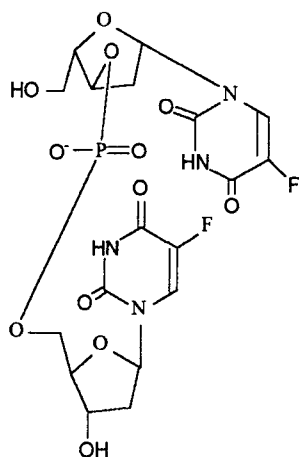
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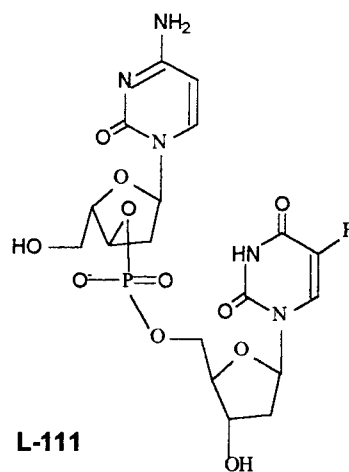
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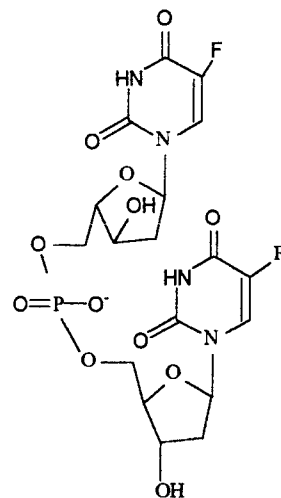
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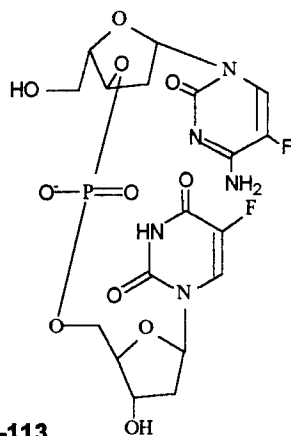
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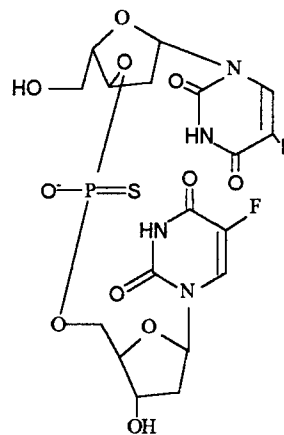
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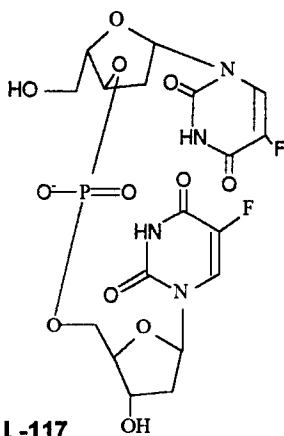
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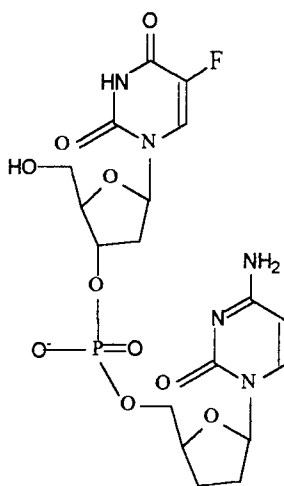
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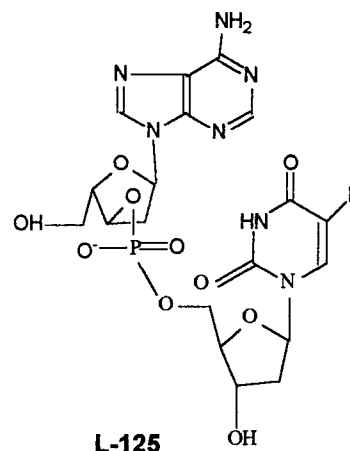
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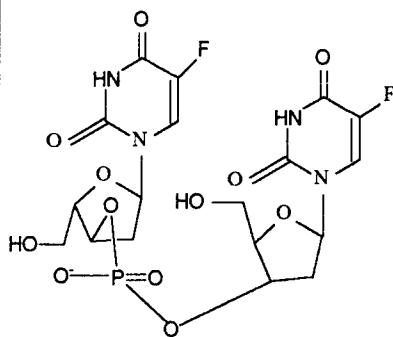
L-124

L-128

L-133

L-138

GCI-1007

GCI 1018

G-1027

GCI 1030

GCI 1032

GCI 1033

GCI 1034

GCI-1036

GCI-1037

GCI-1066

GCI-1069

GCI-1070

GCI-1077 **GCI-1079** **GCI 1085**

GCI 1086 **FUdR** **L-145**

L-147

L-144

L-146
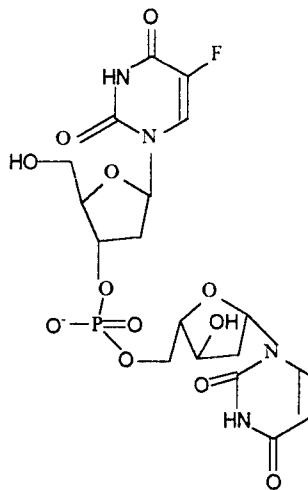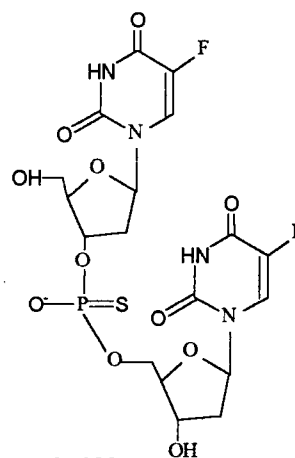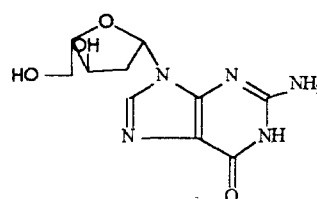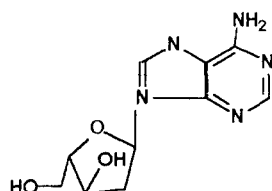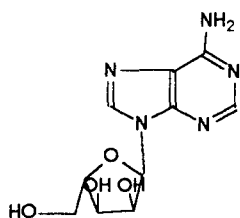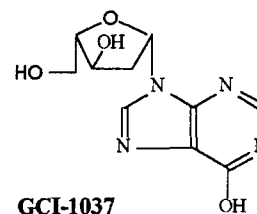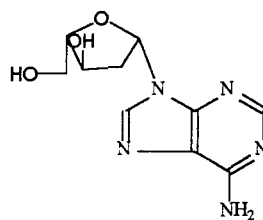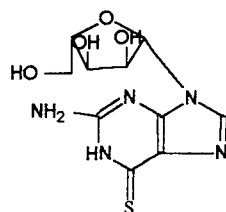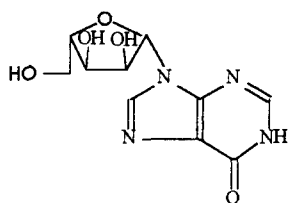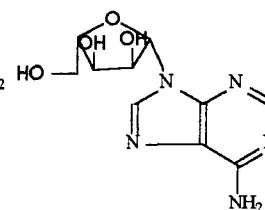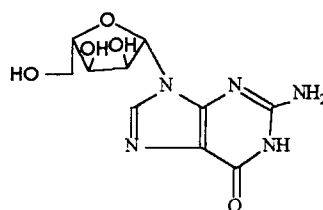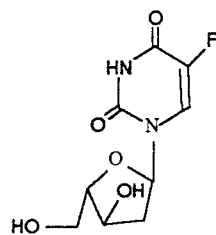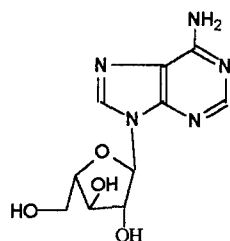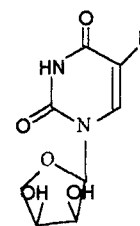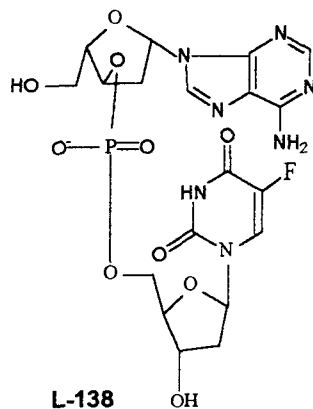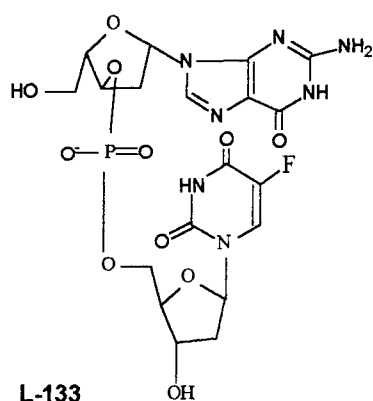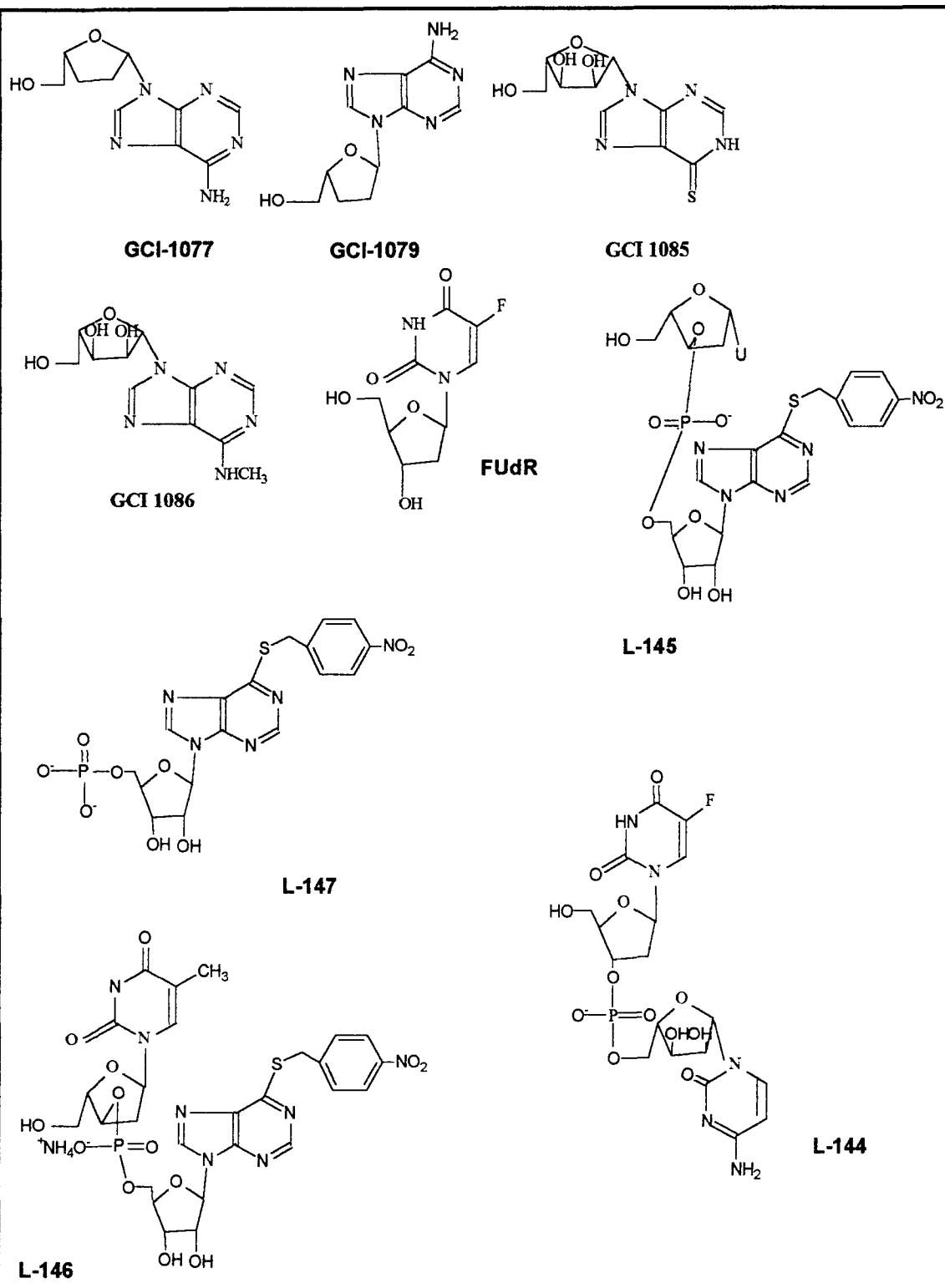
BO1   R$_1$=SATE, R$_2$=H   BO4   R$_1$=H, R$_2$=SATE
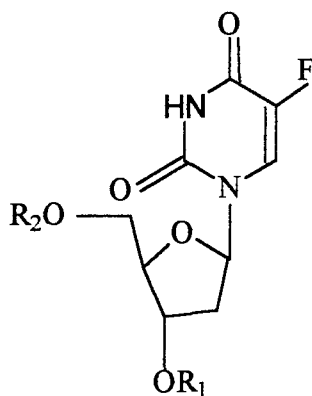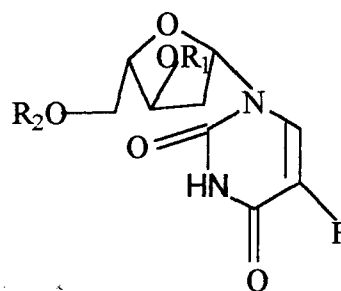
BO2   R$_1$=H, R$_2$=SATE   BO5   R$_1$=R$_2$=SATE
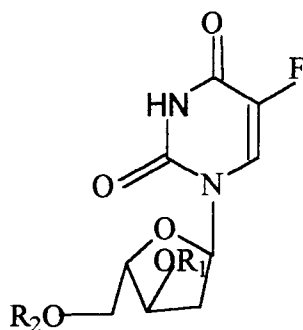
BO3   R$_1$=R$_2$=SATE

BO6   R$_1$=H, R$_2$=SATE

BO7   R$_1$=R$_2$=SATE

SATE: $CH_3COSCH_2CH_2O$

$CH_3COSCH_2CH_2O$

$-P=O$
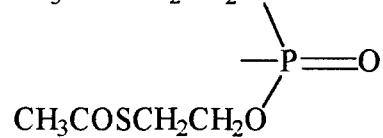
VIII. APPENDIX 2
**The results of the assay of in vitro
activity against P.falciparum infected cells for
selected Lipitek's compounds provided by the
laboratories of Dr. Gero**

Figure 1. Toxicity of L-101 Towards *P. falc*
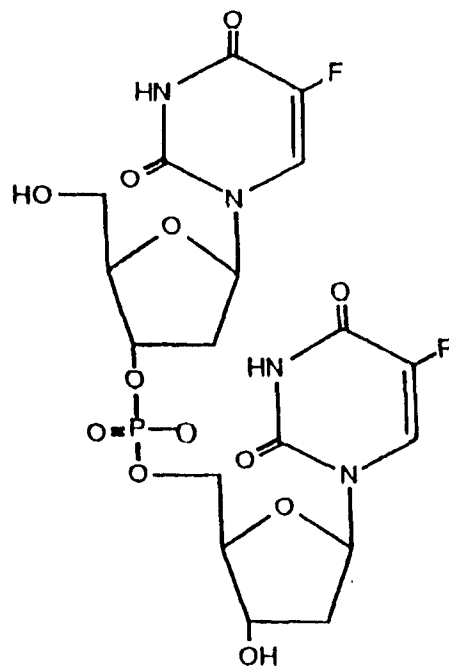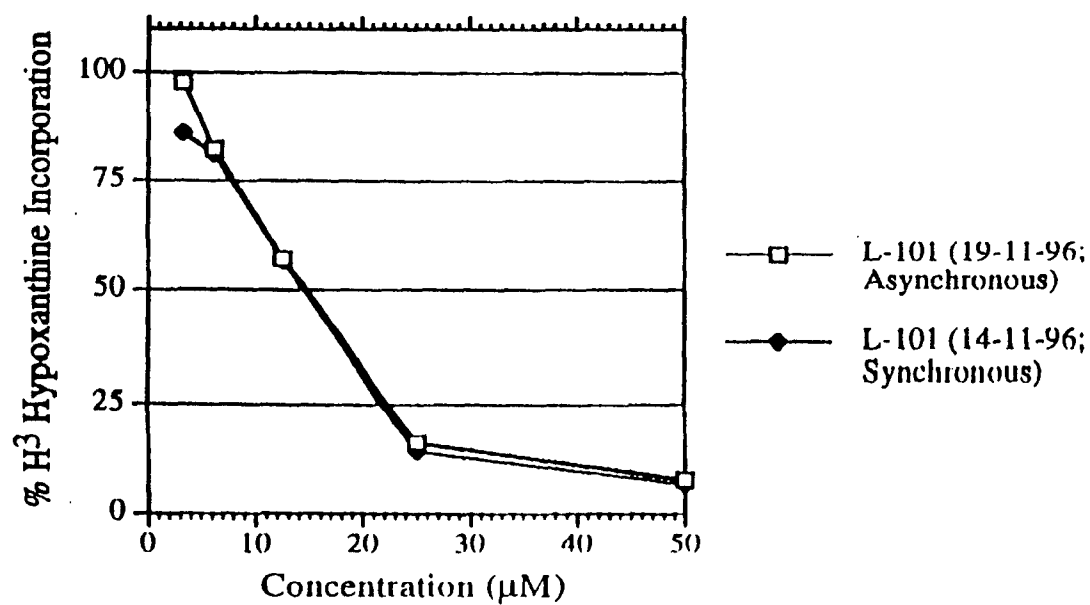
L-101
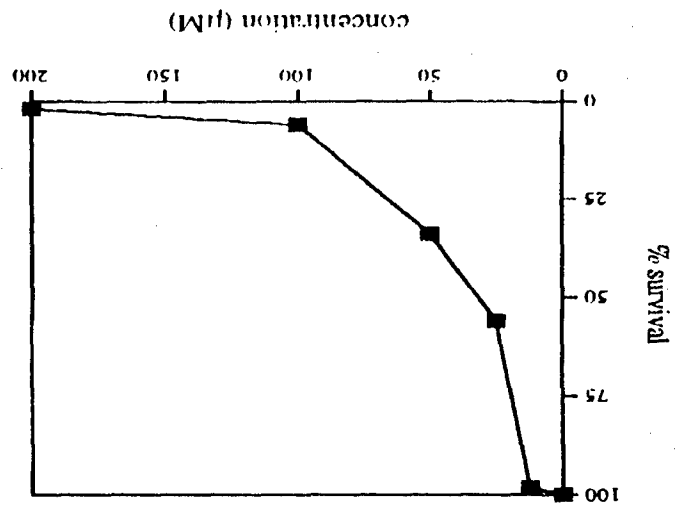
Figure 1a. Survival of cells after treatment with L-101 by microscopic counts of Giemsa stained slides

Figure 2. Toxicity of L-103 Towards *P. falc*

L-103

Figure 3. Toxicity of L-110 Towards *P. falciparum*
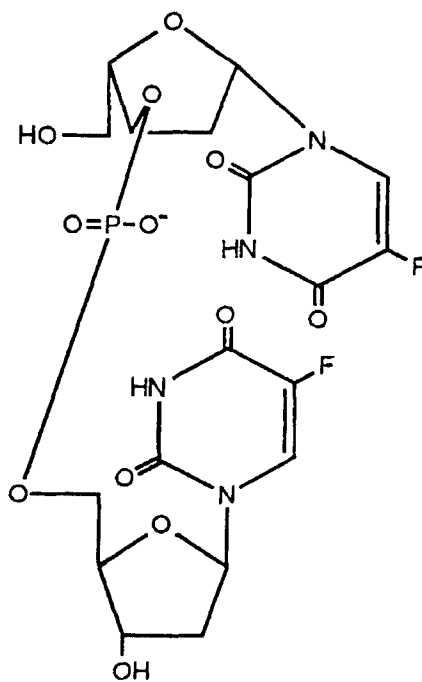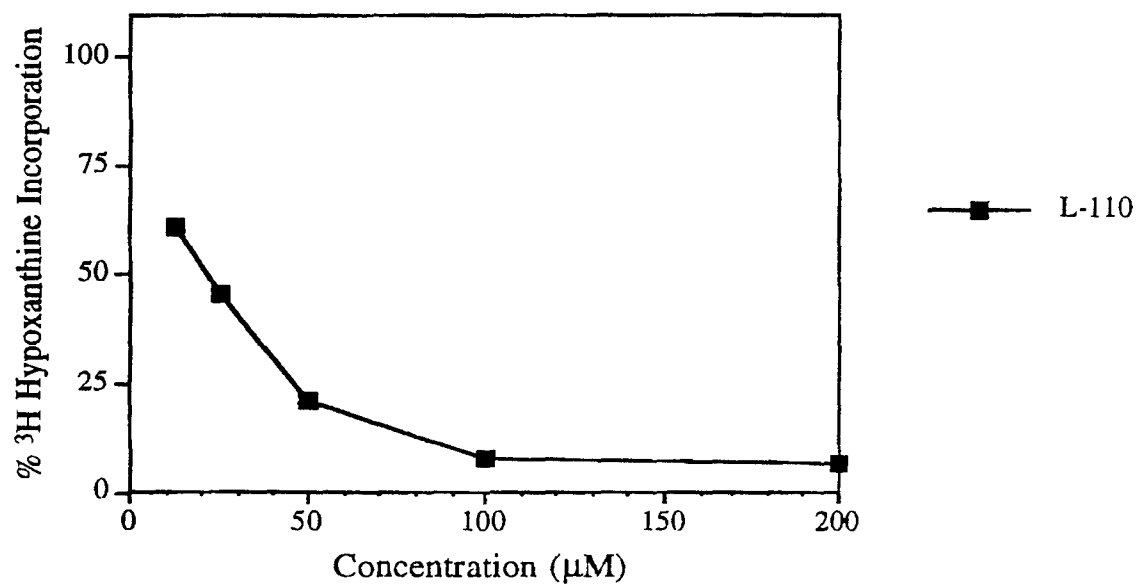
L-110

Figure 4. Toxicity of L-111 Towards *P. falc*
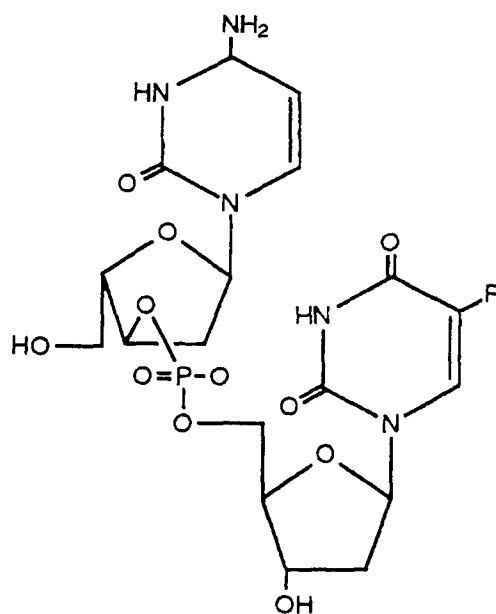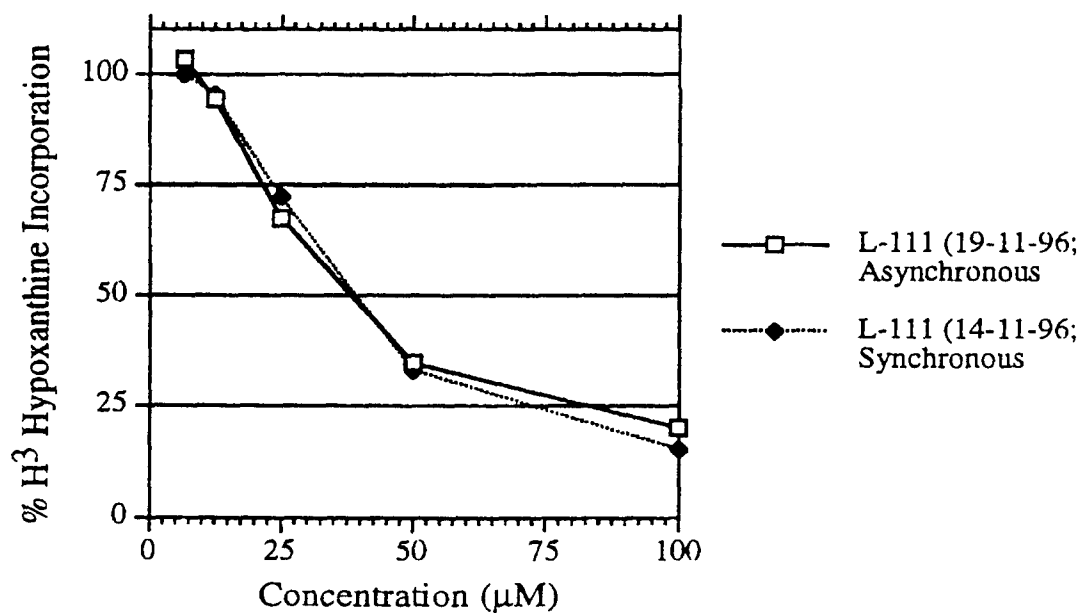
L-111

Figure 4a. Survival of cells after treatment with L-111
by microscopic counts of Giemsa stained slides
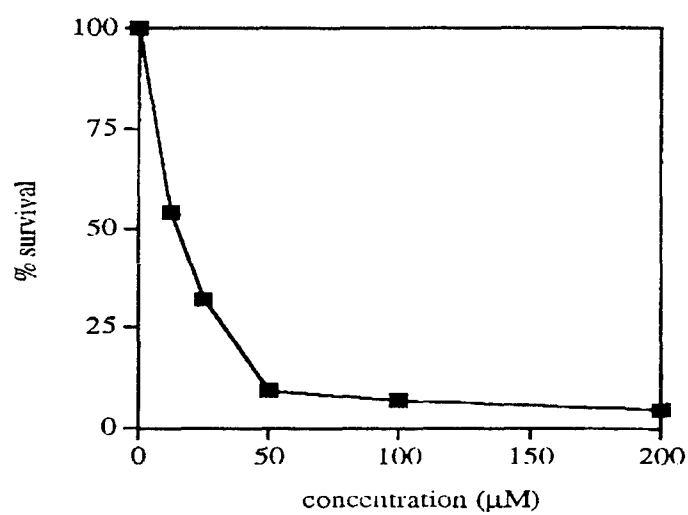
(minimum of 1000 cells counted per concentration)

Figure 5. Toxicity of L-113 Towards *P. falciparum*
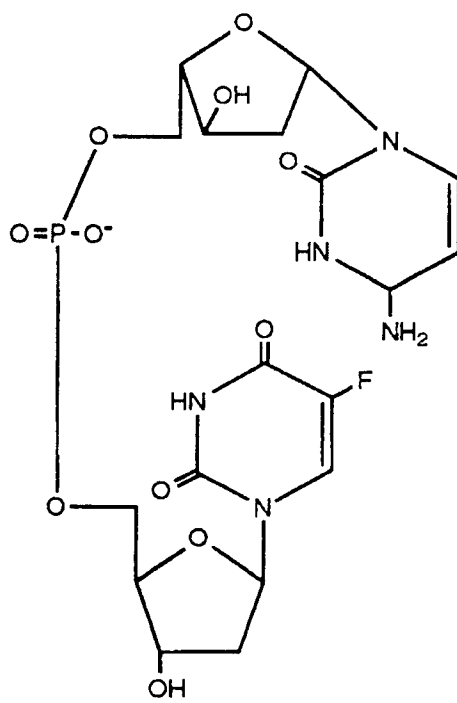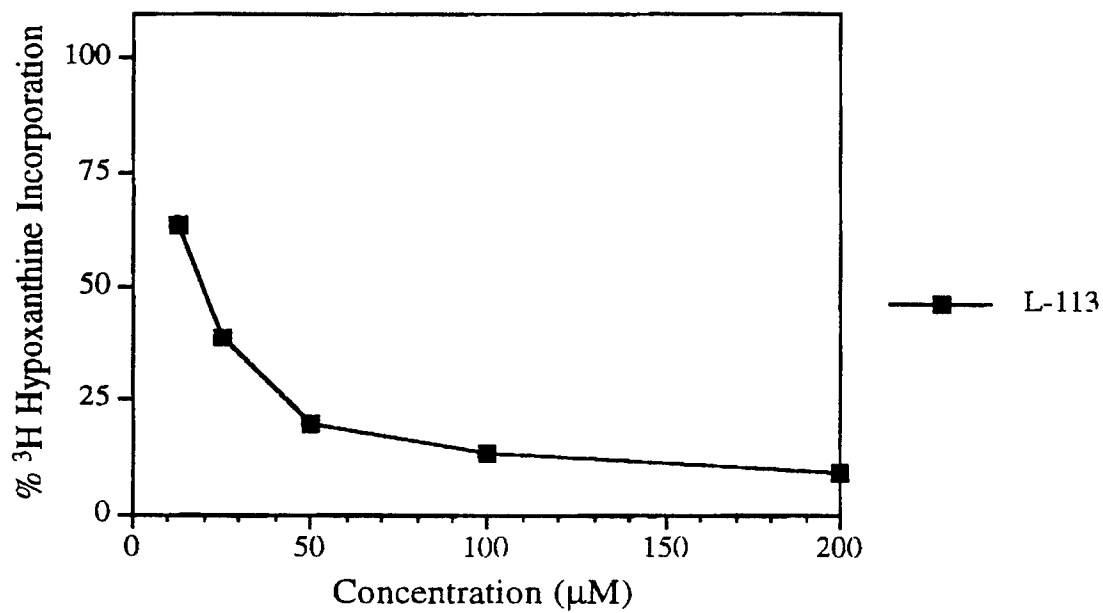
L-113

Figure 6. Toxicity of L-117 Towards *P. falc*
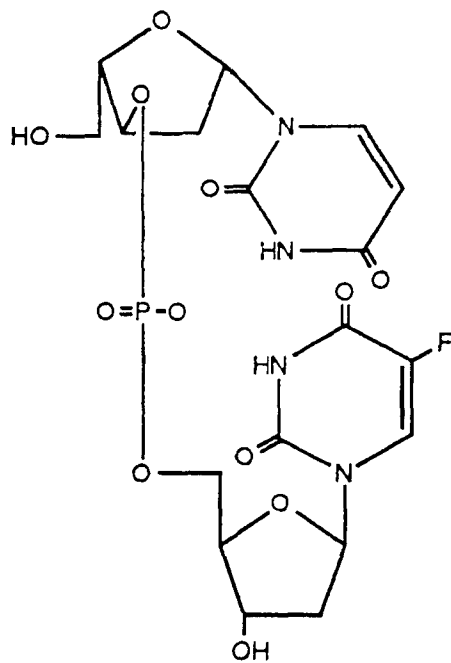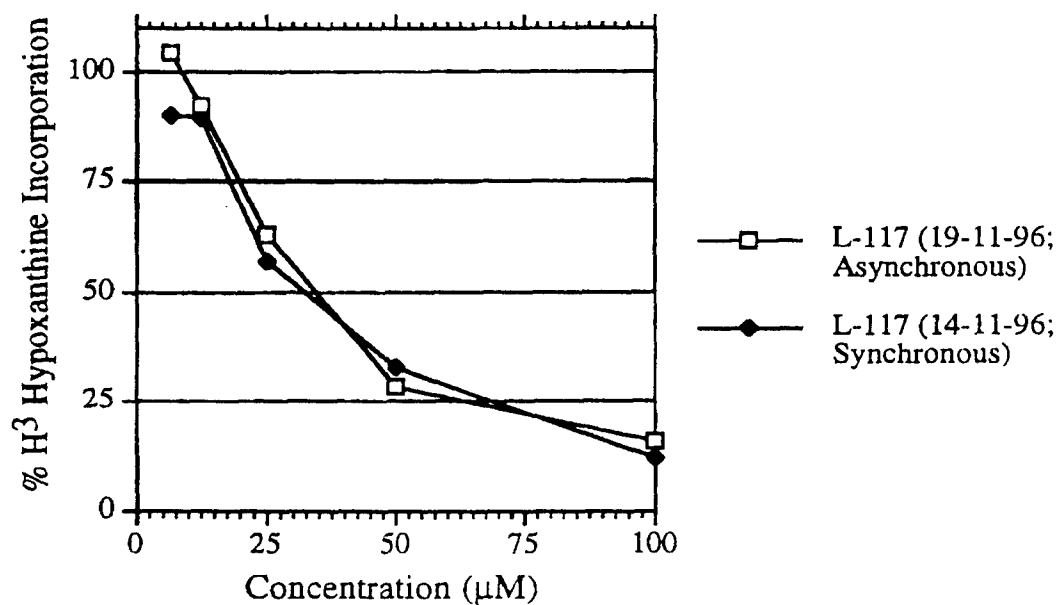
L-117

Figure 6a Survival of cells after treatment with L-117
by microscopic counts of Giemsa stained slides
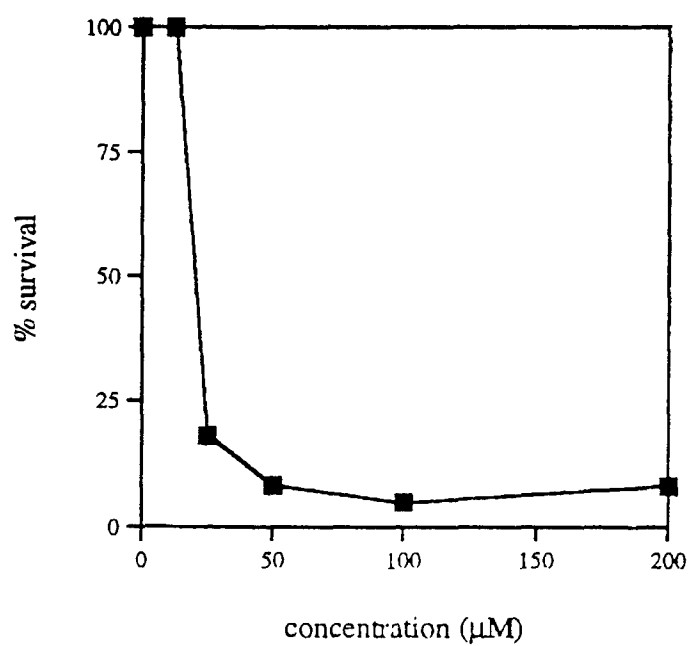
concentration (μM)

(minimum of 1000 cells counted per concentration)

FIGURE 7. Toxicity of L-109 Against *P. falc*
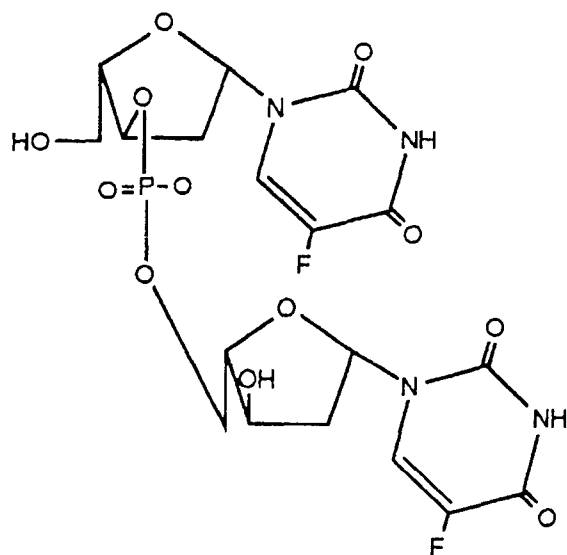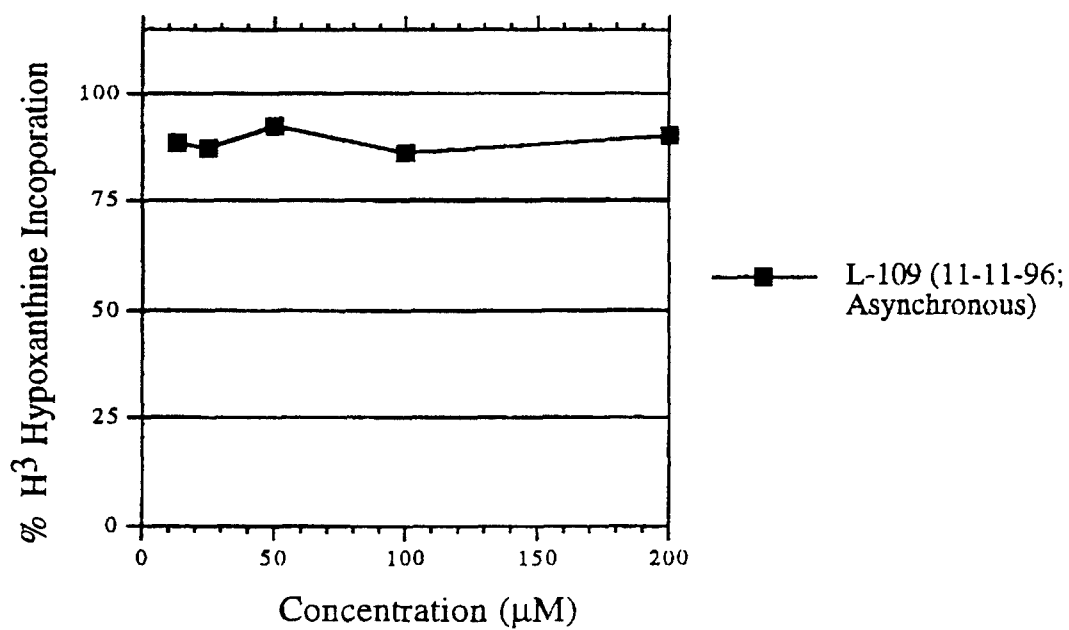
L-109

Figure 8.   Toxicity of L-125 Towards *P. falciparum*

L-125

Figure 9. Toxicity of L-128 Towards *P. falciparum*

L-128

L-128
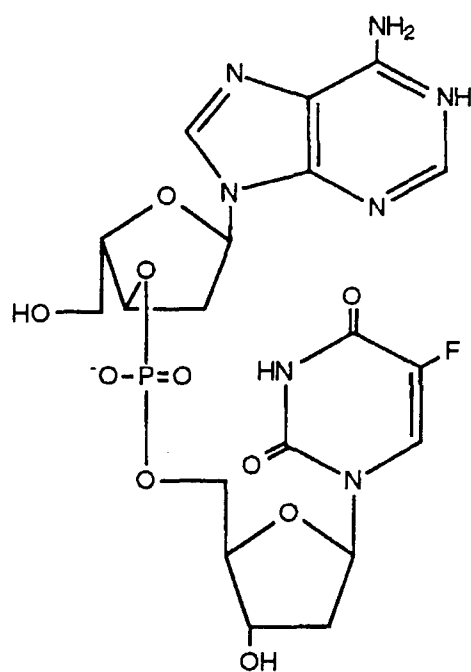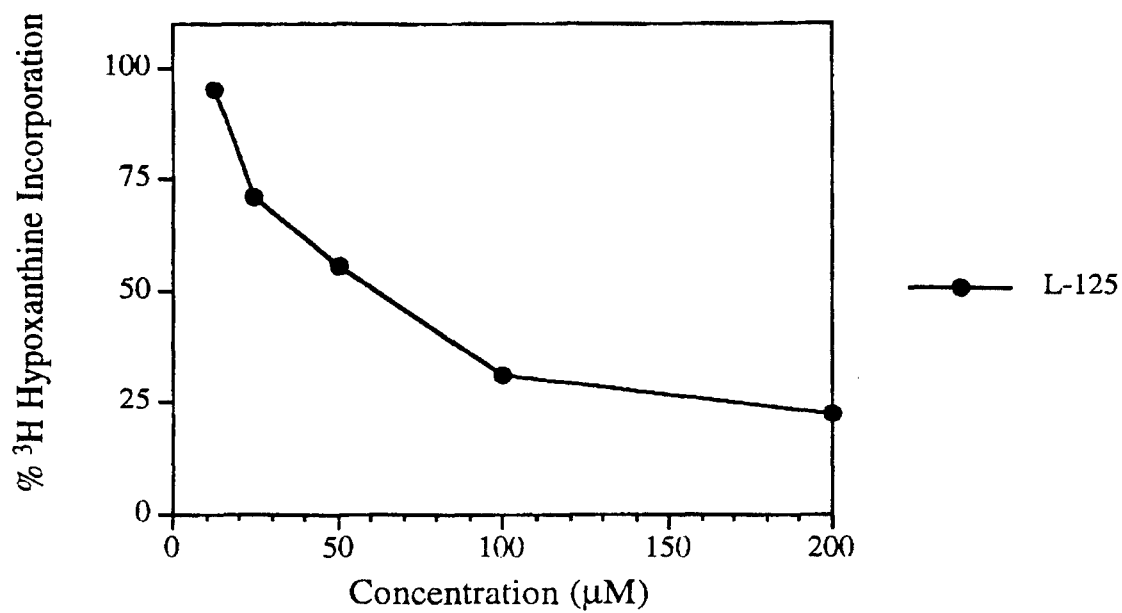
Figure 10. Toxicity of L-133 Towards *P. falc*
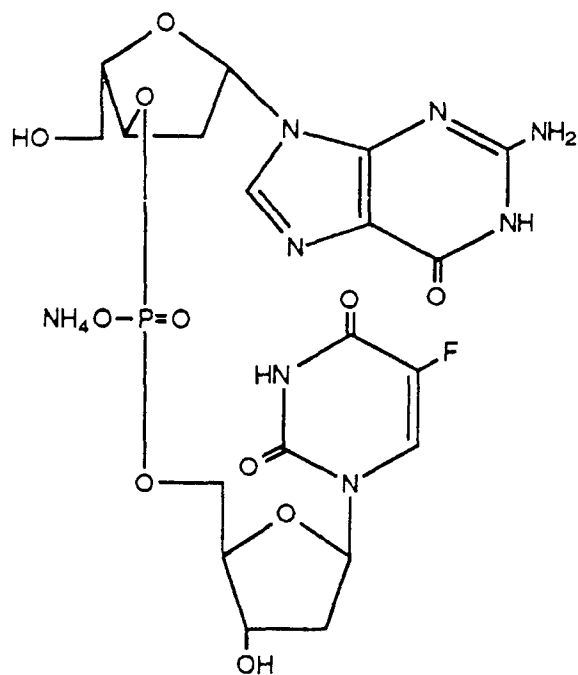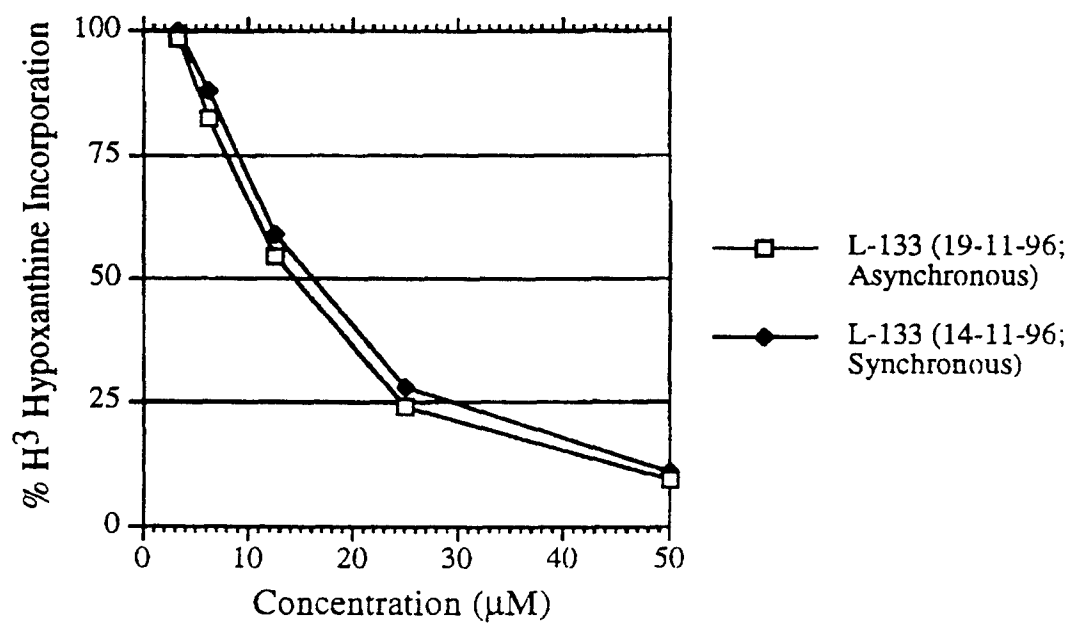
L-133

Figure 10a.   Survival of cells after treatment with L-133
by microscopic counts of Giemsa stained slides

concentration (µM)

(minimum of 1000 cells counted per concentration)

Figure 11. Toxicity of L-138 Towards *P. falc*

- □ — L-138 (19-11-96; Asynchronous)
- ◆ — L-138 (14-11-96; Sunchronous)

L-138
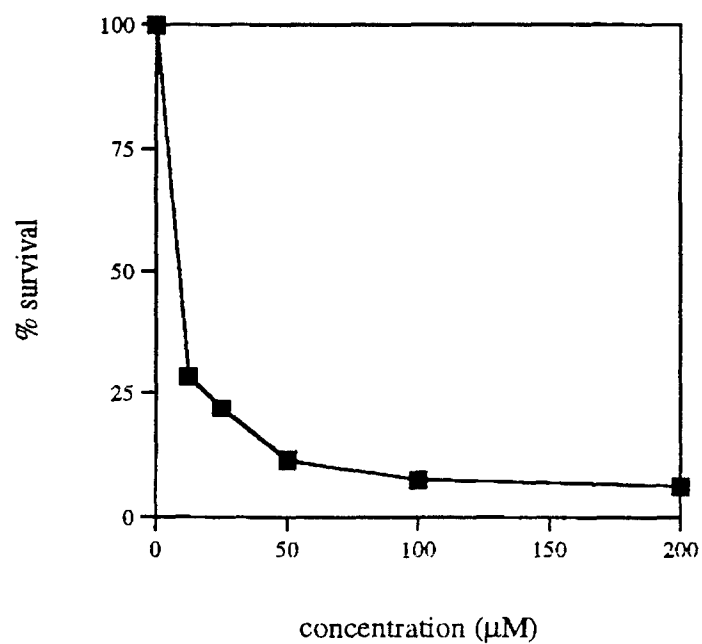
Figure 12. Toxicity of L-144 Towards *P. falciparum*
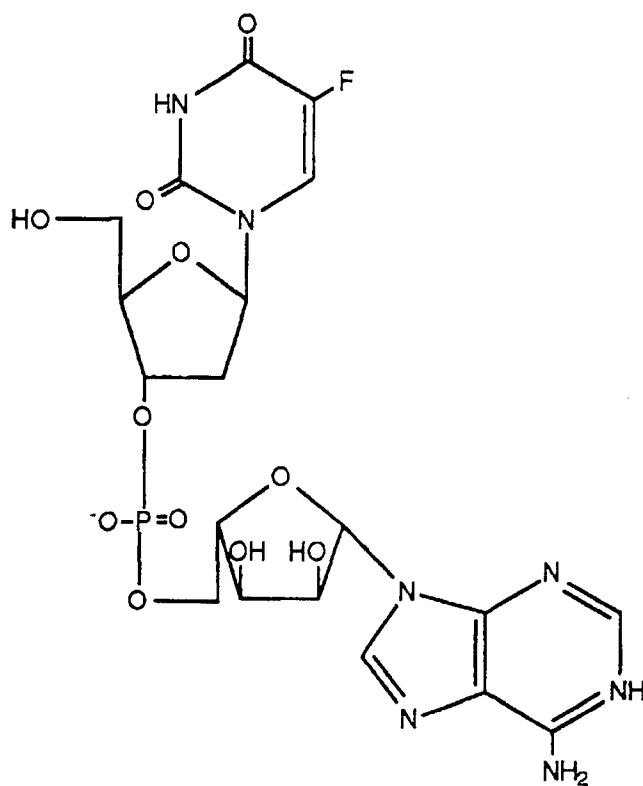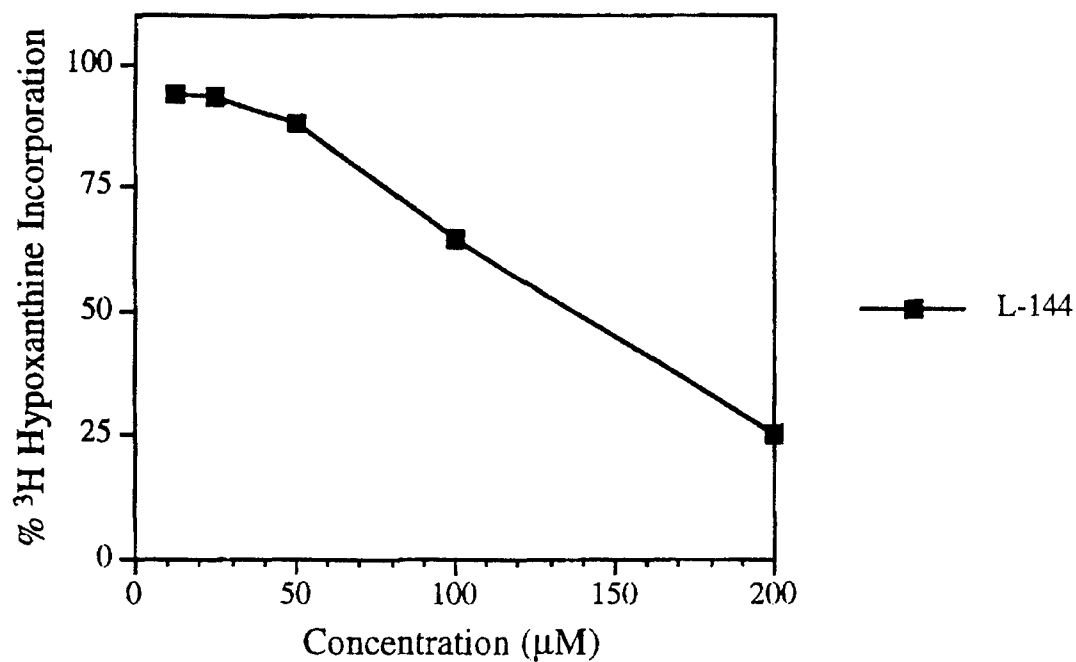
L-144

Figure 13. Foxicity of GCI-1007 Towards *P. falc*
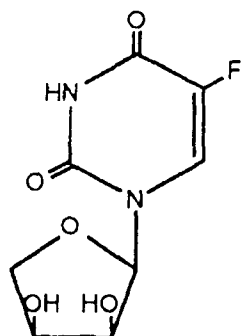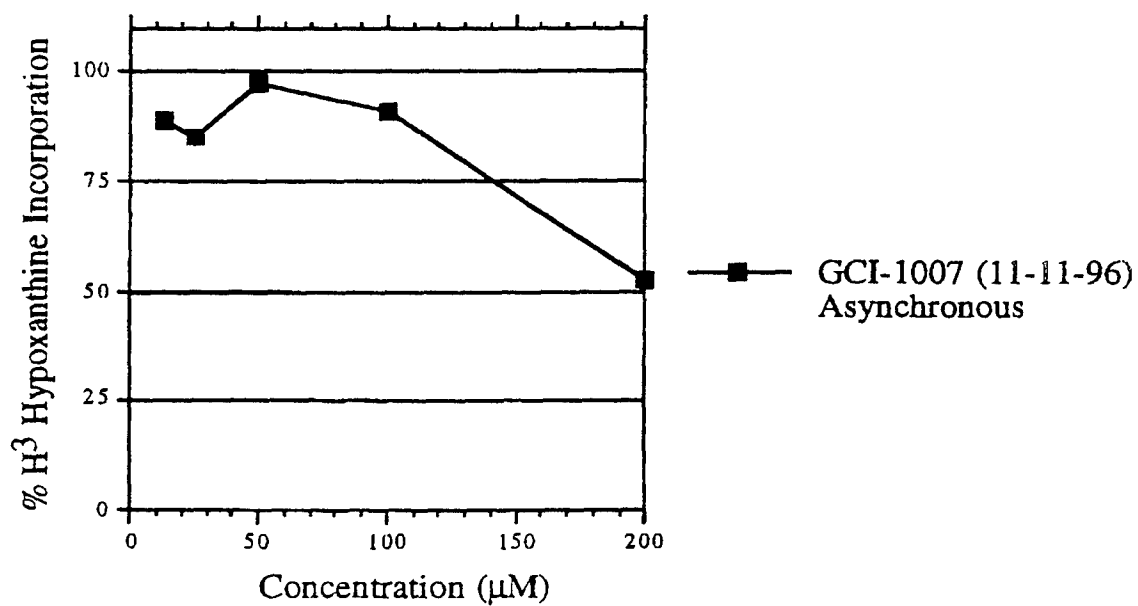
GCI-1007

Figure 14. Toxicity of GCI-1027 towards *P. falciparum*
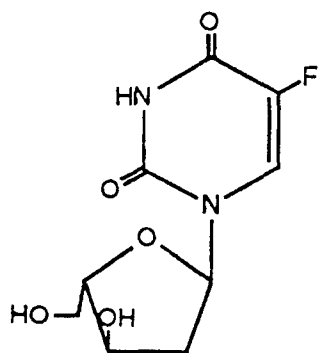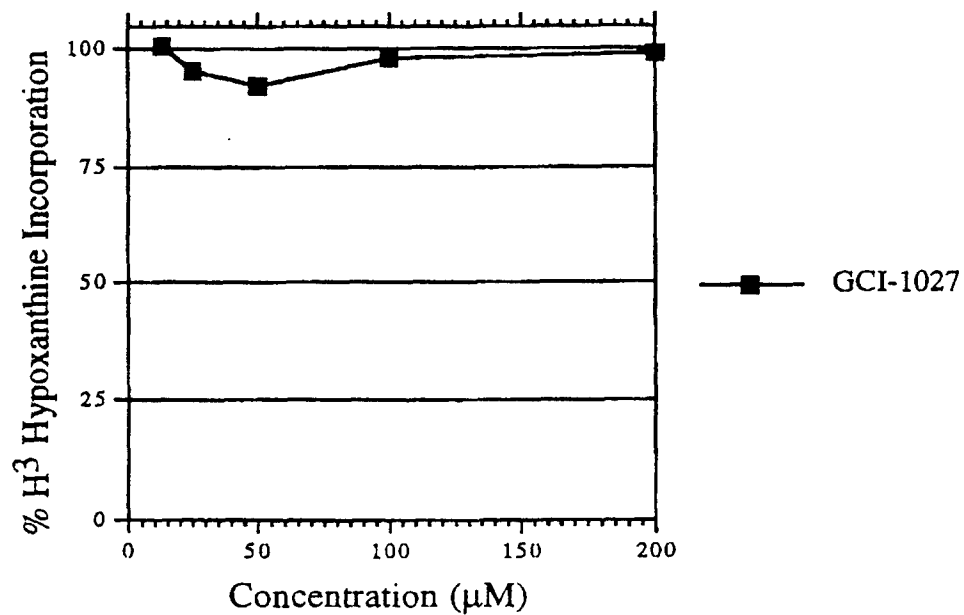
GCI-1027

Figure 15. Toxicity of B01 Towards *P. falciparum*
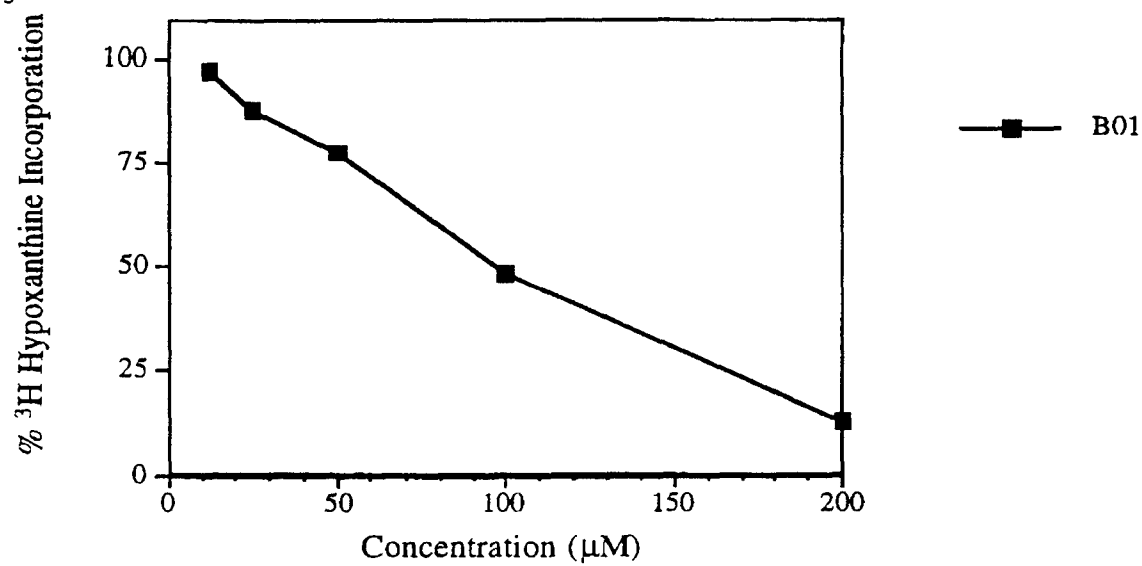
Figure 16. Toxicity of B02 Towards *P. falciparum*
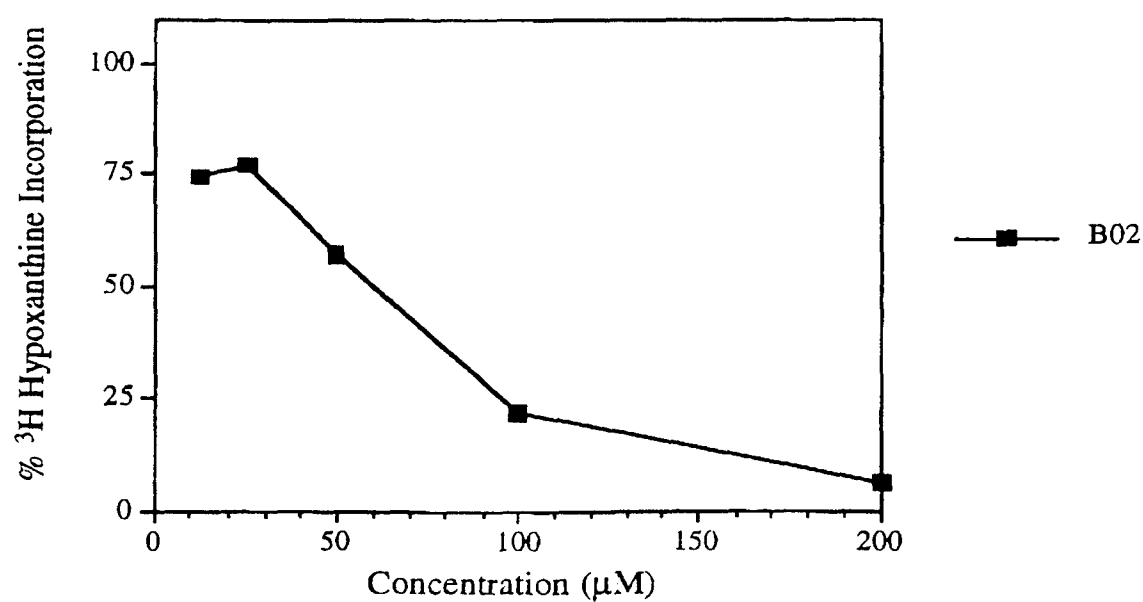
Figure 17.   Toxicity of B03 Towards *P. falciparum*
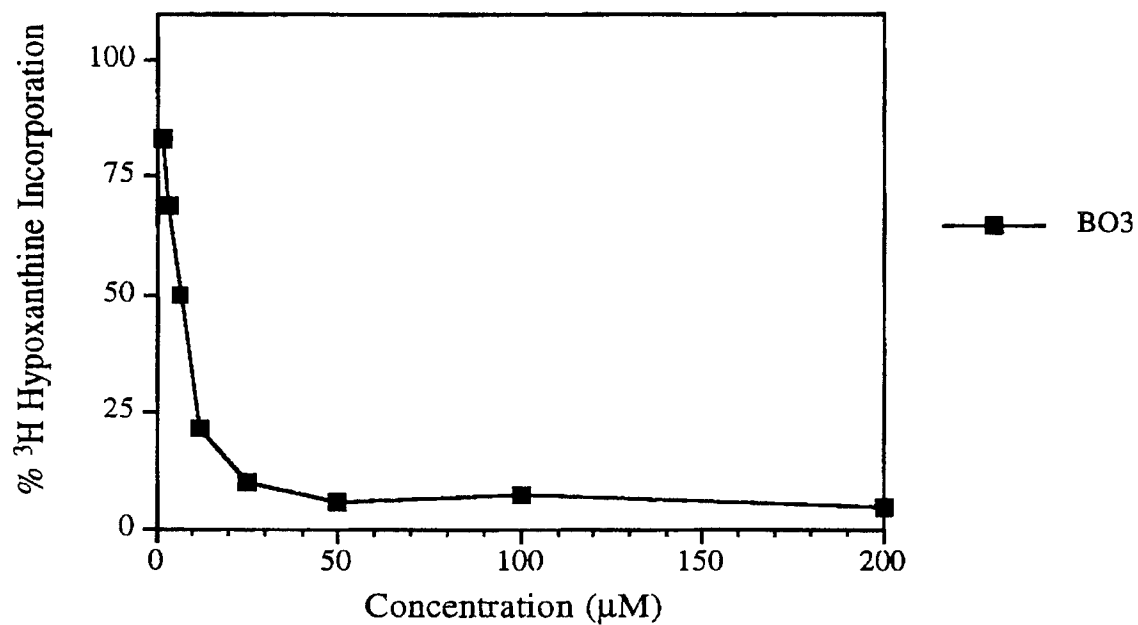
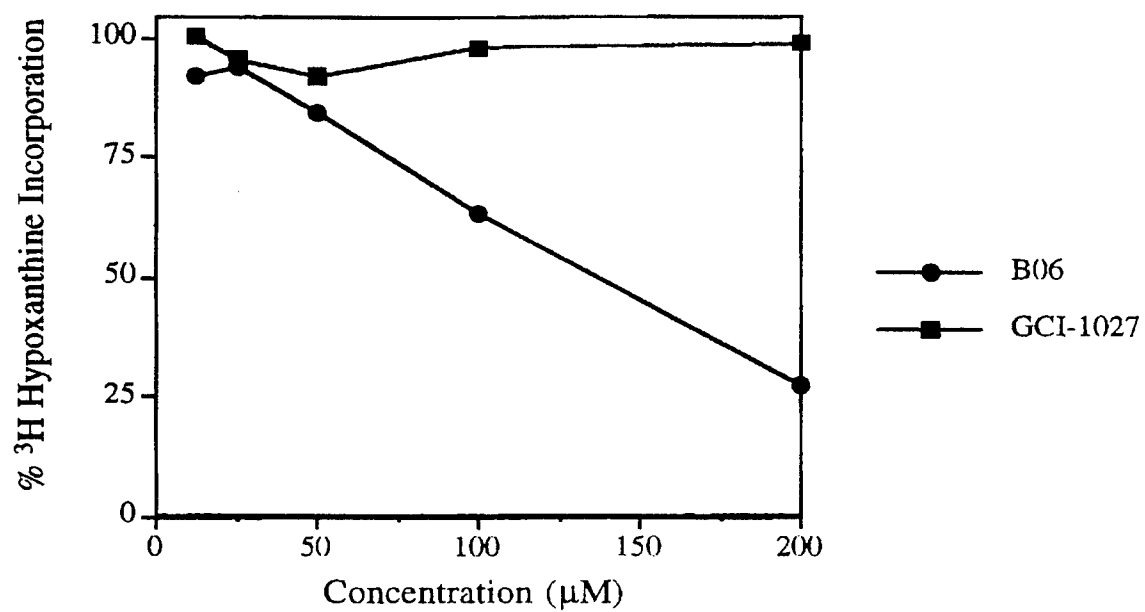

Figure 18.   Toxicity of B04 Towards *P. falciparum*
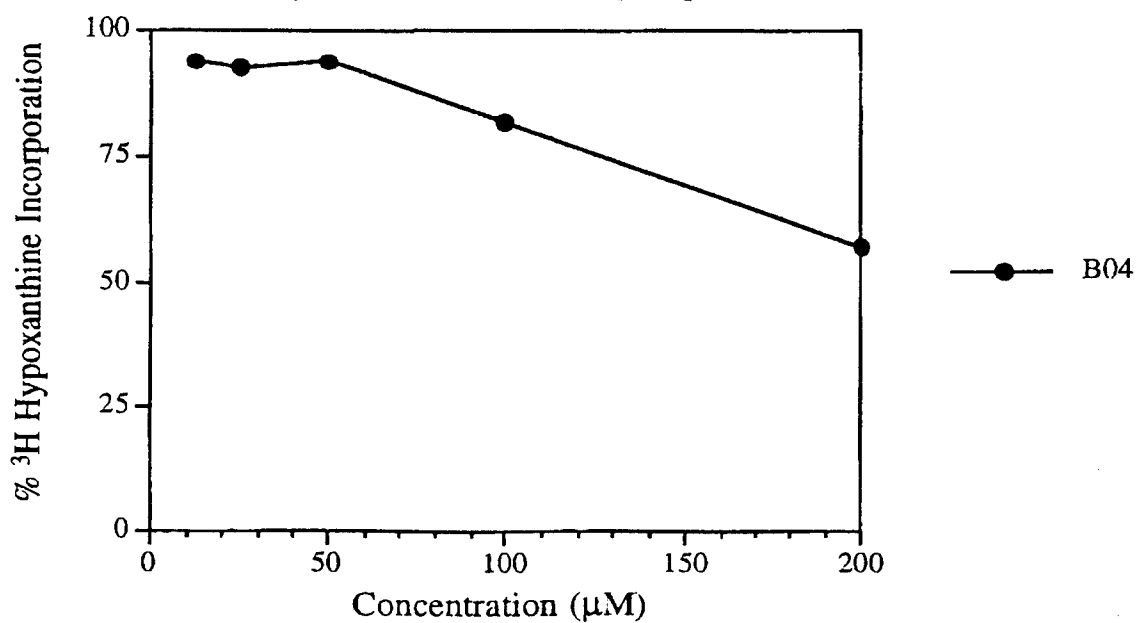
Figure 19. Toxicity of B06 Towards *P. falciparum*
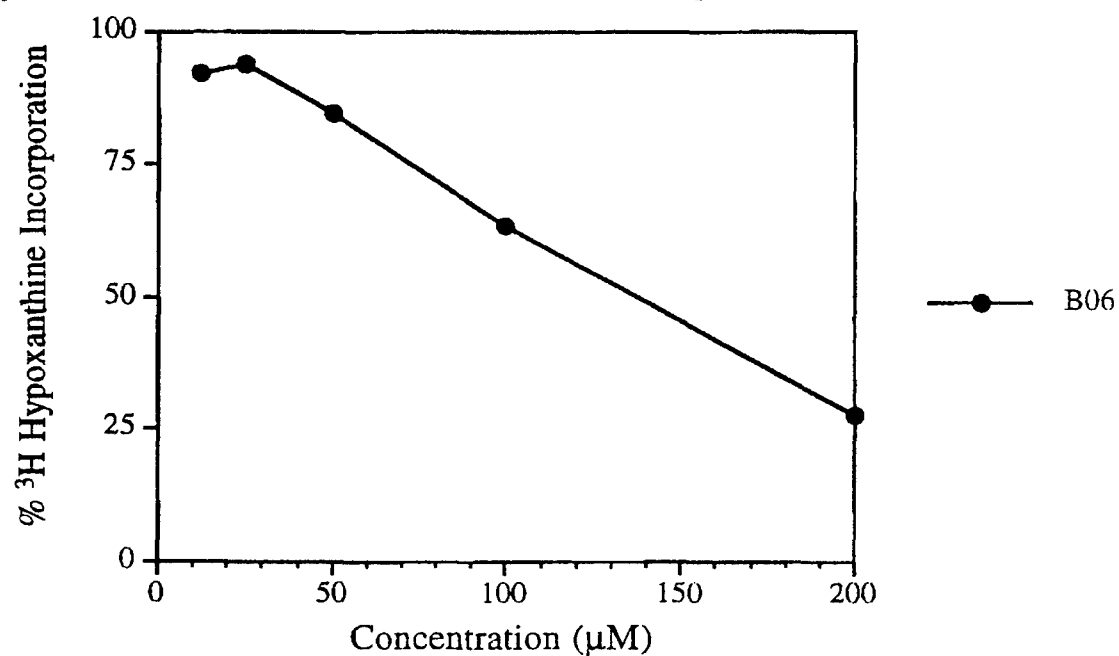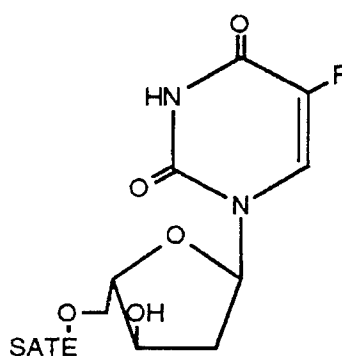

Figure 20. Comparative Toxicity of GCI-1027 & B06
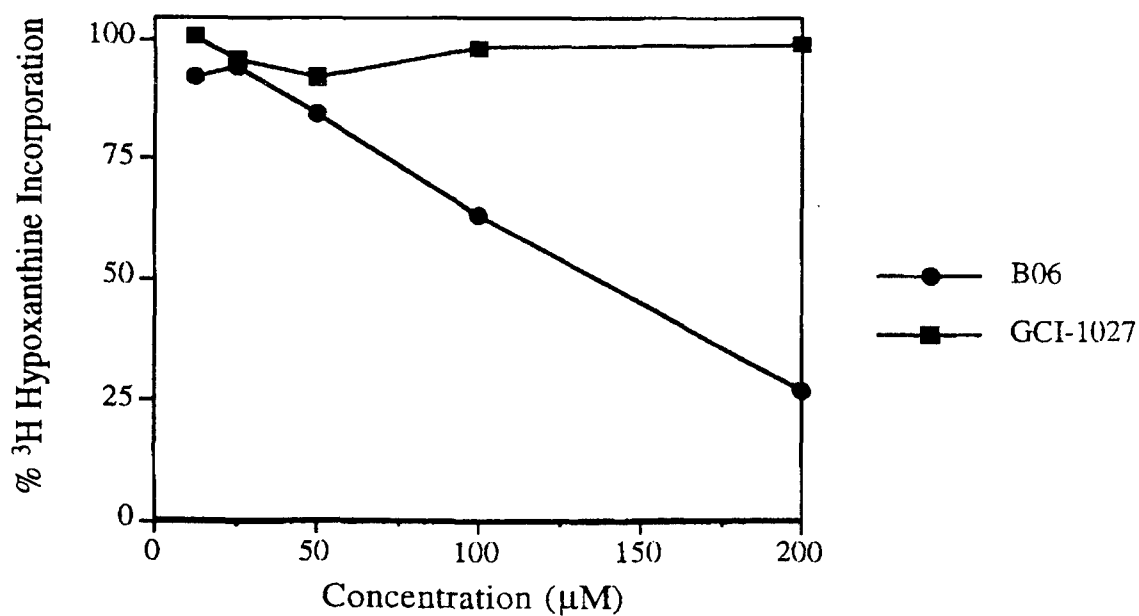
Figure 21.Comparative Toxicity of GCI-1027 & B06

GCI-1027

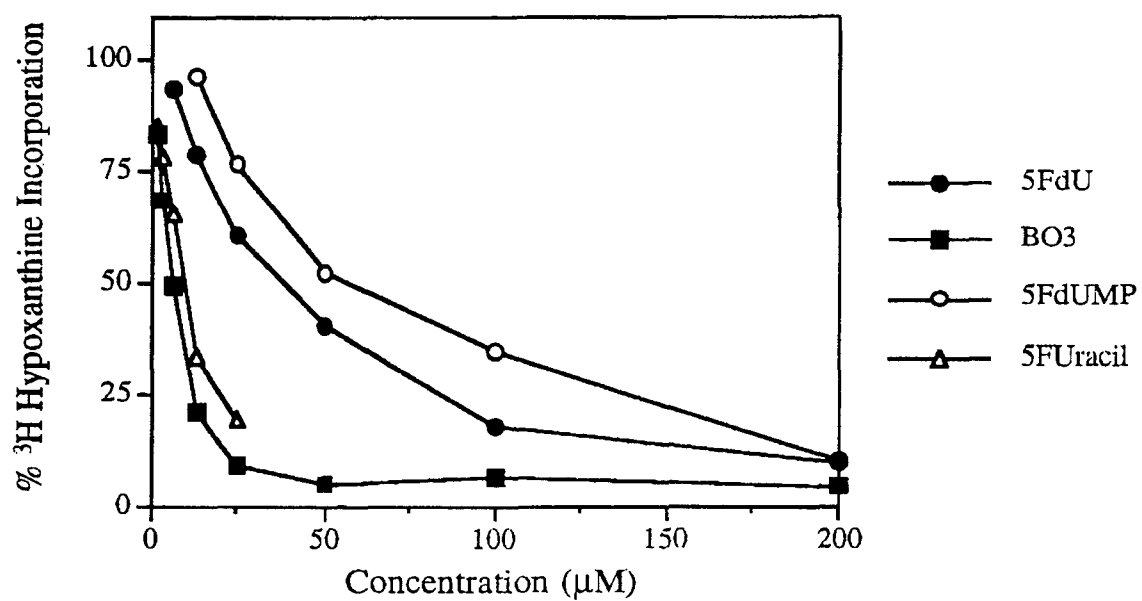BO6
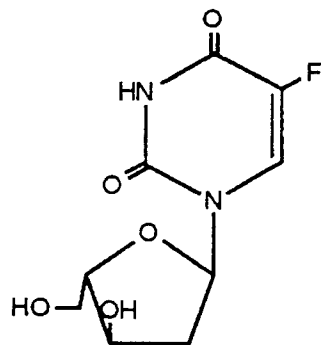
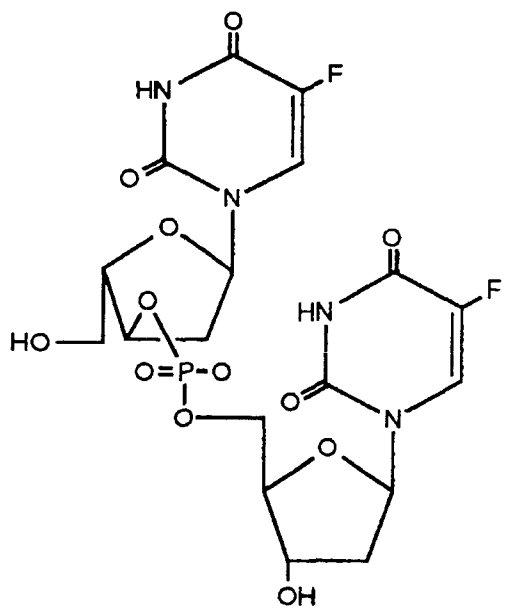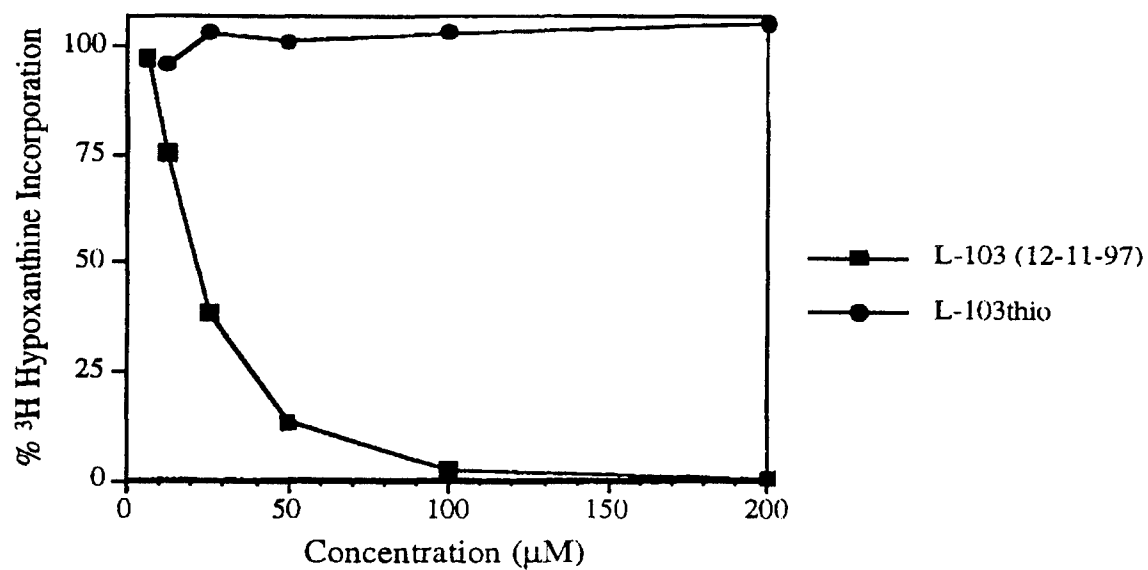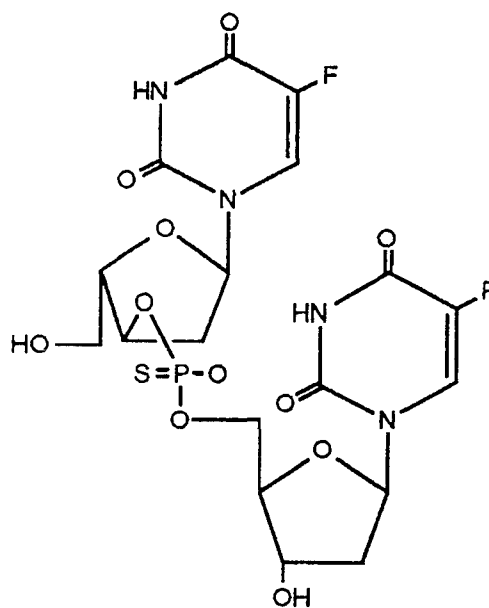Figure 22. Comparative toxicity of 5' fluorouracil
& other related compounds

Figure 23. Comparative Toxicity of L-138 & L-125

L-138

L-125

Figure 24 Comparative Toxicity of L-138 & L-144

L-138

L-144

Figure 25. Comparative Toxicity of L-103 & L-103thio
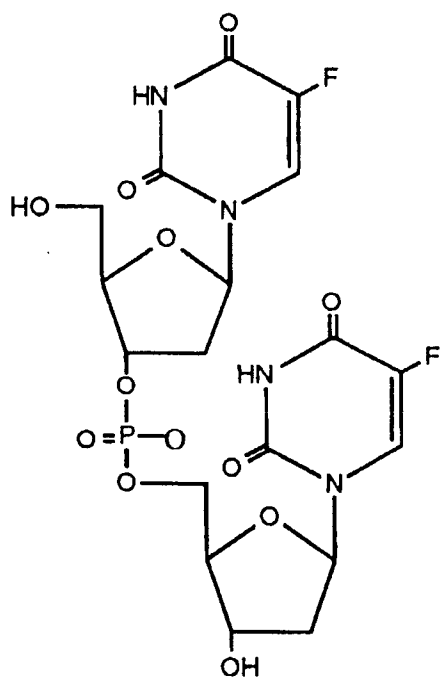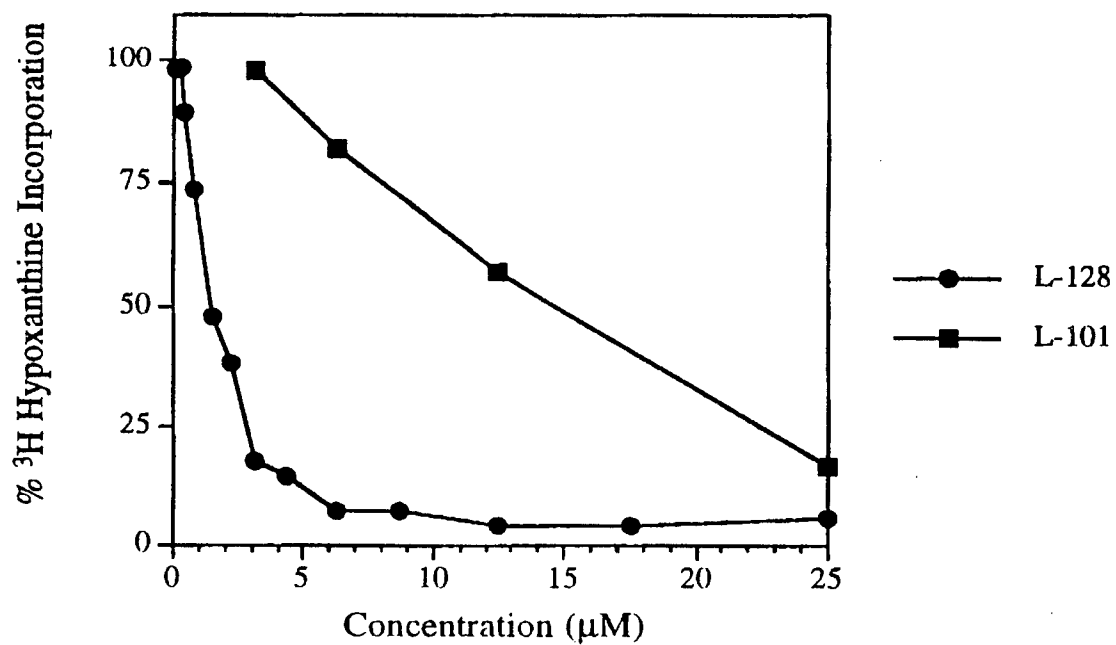
L-103

L-103thio

Figure 26.   Comparative Toxicity of L-101 & L-128 (L-101 thio)

L-101   L-128 (L-101thio)
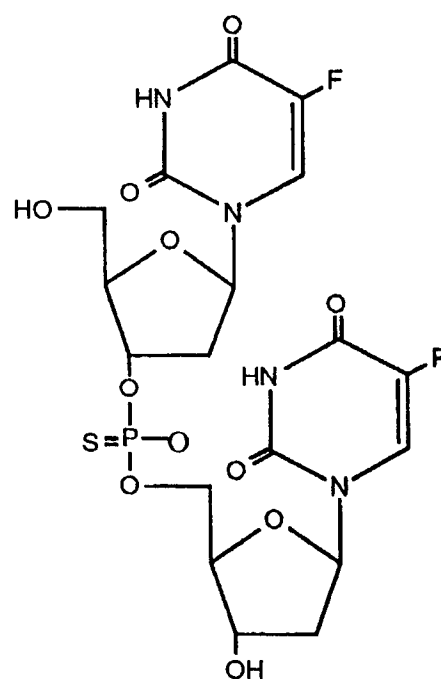
Figure 27. Toxicity of L-117 thio Towards *P. falciparum*

L-117thio

Figure 28. Comparative Toxicity of L-117 & L-117 thio
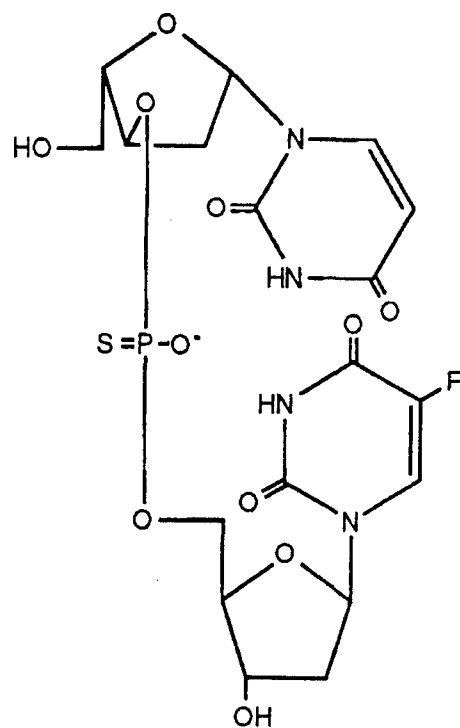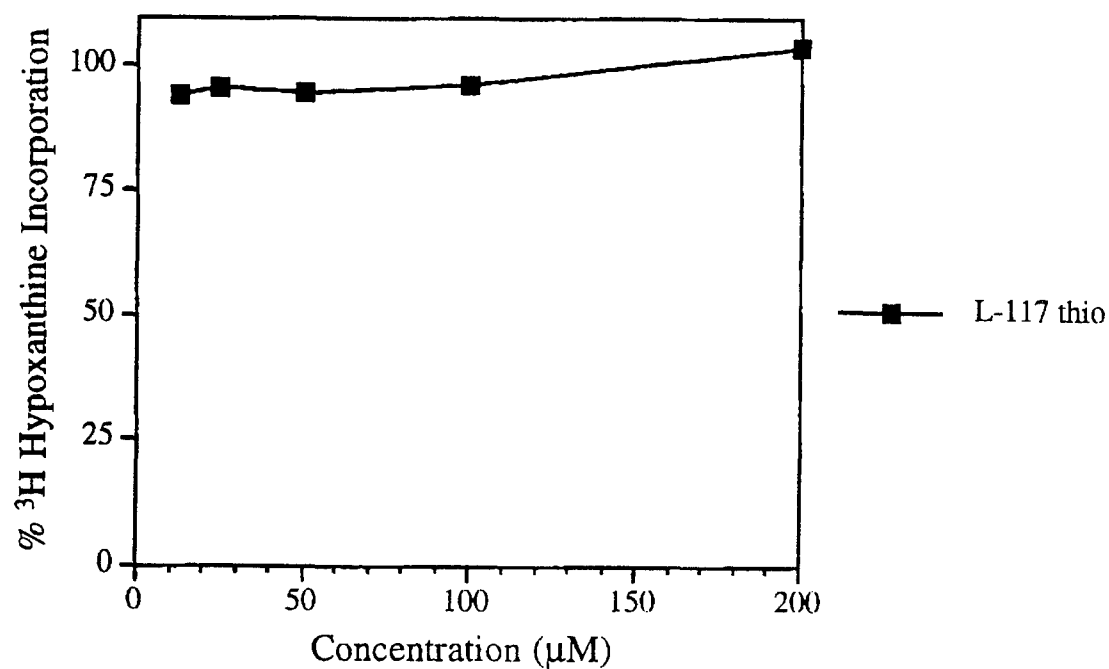
L-117

L-117thio

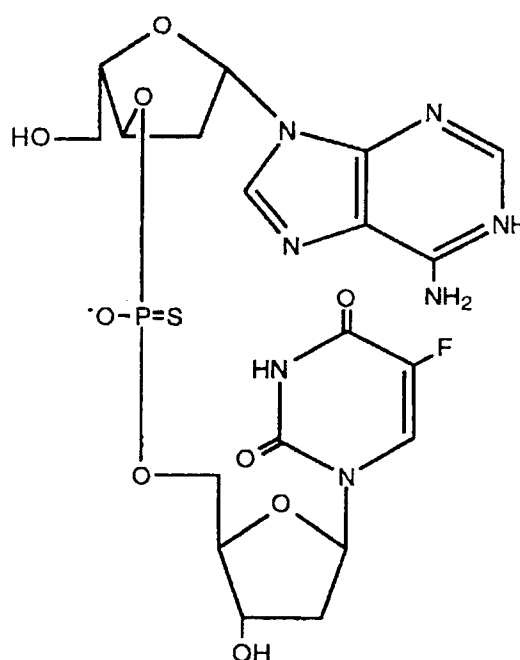Figure 29. Comparative Toxicity of L-138 & L-138 thio
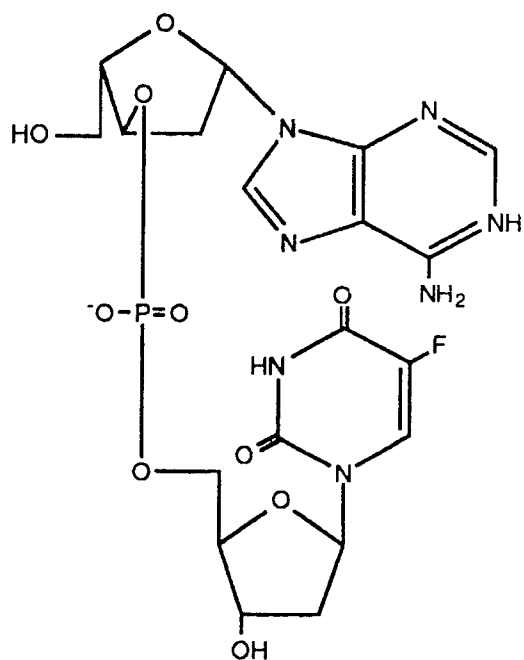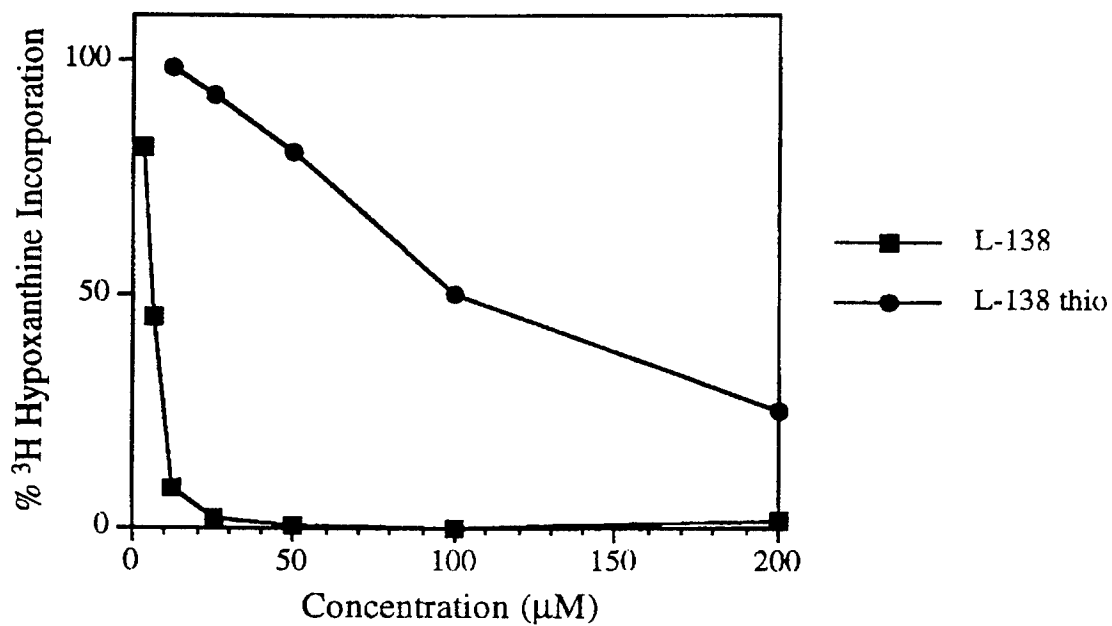
L-138

L-138thio

IX. APPENDIX 3

The results of the assay of *in vitro* activity against *P.falciparum* infected cells for selected Lipitek's compounds provided by the U.S. Army Antimalarial Test Program

INVITRO FALCIPARUM SCREEN - DATA SUMMARY   [D6:RES TO MEF/W2:RES TO CQ,PYR]   28-MAY-97   PAGE NUMBER: 1

FOLLOWING TESTS HAVE BEEN REQUESTED BY : SCOVILL

SUBMITTER REQUESTER	SUBM CODE	VRNO	BN	QOH	PARASITE	IC50	EXP DATE	CTRL DRUG CTRL IC50	CHEMICAL NAME
GOROVITS, D SCOVILL	L-103A	281009	BN86950	0	D6	18849.789	31-MAR-97	CHLOROQUIN 4.394	[ALPHA-L,BETA-D]-FUDR,METHOXY
GOROVITS, D	L-103A	281009	BN86950	0	W2	8423.736	31-MAR-97	CHLOROQUIN 59.922	[ALPHA-L,BETA-D]-FUDR,METHOXY
GOROVITS, D	L-122	281010	BN87037	0	D6	>50000	27-MAR-97	CHLOROQUIN 2.517	[ALPHA-L,BETA-D]-FUDR, 3'-3'
GOROVITS, D	L-122	281010	BN87037	0	W2	>50000	27-MAR-97	CHLOROQUIN 55.043	[ALPHA-L,BETA-D]-FUDR, 3'-3'
GOROVITS, D	L-138	281011	BN87073	0	D6	1723.834	27-MAR-97	CHLOROQUIN 2.517	BETA-L-DA,BETA-D-FUDR
GOROVITS, D	L-138	281011	BN87073	0	D6	1542.629	21-APR-97	CHLOROQUIN 3.830	BETA-L-DA,BETA-D-FUDR
GOROVITS, D	L-138	281011	BN87073	0	W2	995.194	21-APR-97	CHLOROQUIN 5.023	BETA-L-DA,BETA-D-FUDR
GOROVITS, D	L-138	281011	BN87073	0	W2	613.758	27-MAR-97	CHLOROQUIN 55.043	BETA-L-DA,BETA-D-FUDR
GOROVITS, D	L-110	281012	BN86978	0	D6	1674.008	31-MAR-97	CHLOROQUIN 4.394	[BETA-L,BETA-D]-FUDR
GOROVITS, D	L-110	281012	BN86978	0	D6	1516.885	21-APR-97	CHLOROQUIN 3.830	[BETA-L,BETA-D]-FUDR
GOROVITS, D	L-110	281012	BN86978	0	W2	262.250	31-MAR-97	CHLOROQUIN 59.922	[BETA-L,BETA-D]-FUDR
GOROVITS, D	L-110	281012	BN86978	0	W2	1592.425	21-APR-97	CHLOROQUIN 5.023	[BETA-L,BETA-D]-FUDR
GOROVITS, D	L-111	281013	BN86987	0	D6	9045.880	27-MAR-97	CHLOROQUIN 59.922	ALPHA-L-DC,BETA-D-FUDR
GOROVITS, D	L-111	281013	BN86987	0	D6	5574.767	21-APR-97	CHLOROQUIN 2.517	ALPHA-L-DC,BETA-D-FUDR
GOROVITS, D	L-111	281013	BN86987	0	W2	5253.198	27-MAR-97	CHLOROQUIN 3.830	ALPHA-L-DC,BETA-D-FUDR
GOROVITS, D	L-111	281013	BN86987	0	W2	5058.042	21-APR-97	CHLOROQUIN 55.043	ALPHA-L-DC,BETA-D-FUDR
GOROVITS, D	L-112	281014	BN86996	0	D6	1615.964	27-MAR-97	CHLOROQUIN 5.023	[ALPHA-L,BETA-D]-FUDR,5'-5'
GOROVITS, D	L-112	281014	BN86996	0	D6	639.354	21-APR-97	CHLOROQUIN 2.517 3.830	[ALPHA-L,BETA-D]-FUDR,5'-5'

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INVITRO FALCIPARUM SCREEN - DATA SUMMARY   28-MAY-97   PAGE NUMBER: 2

[D6:RES TO MEF/W2:RES TO CQ,PYR]

SUBMITTER REQUESTER	SUBM CODE	WRNO	BN	QOH	PARASITE	IC50	EXP DATE	CTRL DRUG CTRL IC50	CHEMICAL NAME
GOROVITS, D	L-112	281014	BN86996	0	W2	1816.108	21-APR-97	CHLOROQUIN 5.023	[ALPHA-L,BETA-D]-FUDR,5'-5'
GOROVITS, D	L-112	281014	BN86996	0	W2	873.315	27-MAR-97	CHLOROQUIN 55.043	[ALPHA-L,BETA-D]-FUDR,5'-5'
GOROVITS, D	L-103	281015	BN86941	0	D6	4097.443	31-MAR-97	CHLOROQUIN 4.394	[ALPHA-L,BETA-D]-FUDR
GOROVITS, D	L-103	281015	BN86941	0	D6	2355.950	21-APR-97	CHLOROQUIN 3.830	[ALPHA-L,BETA-D]-FUDR
GOROVITS, D	L-103	281015	BN86941	0	W2	2361.897	21-APR-97	CHLOROQUIN 5.023	[ALPHA-L,BETA-D]-FUDR
GOROVITS, D	L-103	281015	BN86941	0	W2	1760.746	31-MAR-97	CHLOROQUIN 59.922	[ALPHA-L,BETA-D]-FUDR
GOROVITS, D	L-101	281016	BN86932	0	D6	1478.660	21-APR-97	CHLOROQUIN 3.830	[BETA-D,BETA-D]-FUDR
GOROVITS, D	L-101	281016	BN86932	0	D6	359.399	31-MAR-97	CHLOROQUIN 4.394	[BETA-D,BETA-D]-FUDR
GOROVITS, D	L-101	281016	BN86932	0	W2	1162.599	21-APR-97	CHLOROQUIN 5.023	[BETA-D,BETA-D]-FUDR
GOROVITS, D	L-101	281016	BN86932	0	W2	541.356	31-MAR-97	CHLOROQUIN 59.922	[BETA-D,BETA-D]-FUDR
GOROVITS, D	L-120	281017	BN87028	0	D6	>50000	27-MAR-97	CHLOROQUIN 2.517	BETA-D-FUDR,ALPHA-L-DDC
GOROVITS, D	L-120	281017	BN87028	0	W2	>50000	27-MAR-97	CHLOROQUIN 55.043	BETA-D-FUDR,ALPHA-L-DDC
GOROVITS, D	L-114	281018	BN87000	0	D6	>50000	27-MAR-97	CHLOROQUIN 2.517	BETA-D-FUDR,BETA-L-DC
GOROVITS, D	L-114	281018	BN87000	0	W2	>50000	27-MAR-97	CHLOROQUIN 55.043	BETA-D-FUDR,BETA-L-DC
GOROVITS, D	L-107	281019	BN86969	0	D6	17036.350	31-MAR-97	CHLOROQUIN 4.394	ALPHA-L-DU, BETA-D-FUDR
GOROVITS, D	L-107	281019	BN86969	0	D6	7691.275	21-APR-97	CHLOROQUIN 3.830	ALPHA-L-DU, BETA-D-FUDR
GOROVITS, D	L-107	281019	BN86969	0	W2	6877.783	21-APR-97	CHLOROQUIN 5.023	ALPHA-L-DU, BETA-D-FUDR
GOROVITS, D	L-107	281019	BN86969	0	W2	5406.865	31-MAR-97	CHLOROQUIN 59.922	ALPHA-L-DU, BETA-D-FUDR
GOROVITS, D	L-124	281020	BN87046	0	D6	>50000	27-MAR-97	CHLOROQUIN 2.517	BETA-D-FUDR,BETA-L-DU
GOROVITS, D	L-124	281020	BN87046	0	W2	>50000	27-MAR-97	CHLOROQUIN 55.043	BETA-D-FUDR,BETA-L-DU

INVITRO FALCIPARUM SCREEN - DATA SUMMARY   [D6:RES TO MEF/W2:RES TO CQ,PYR]   28-MAY-97   PAGE NUMBER: 3

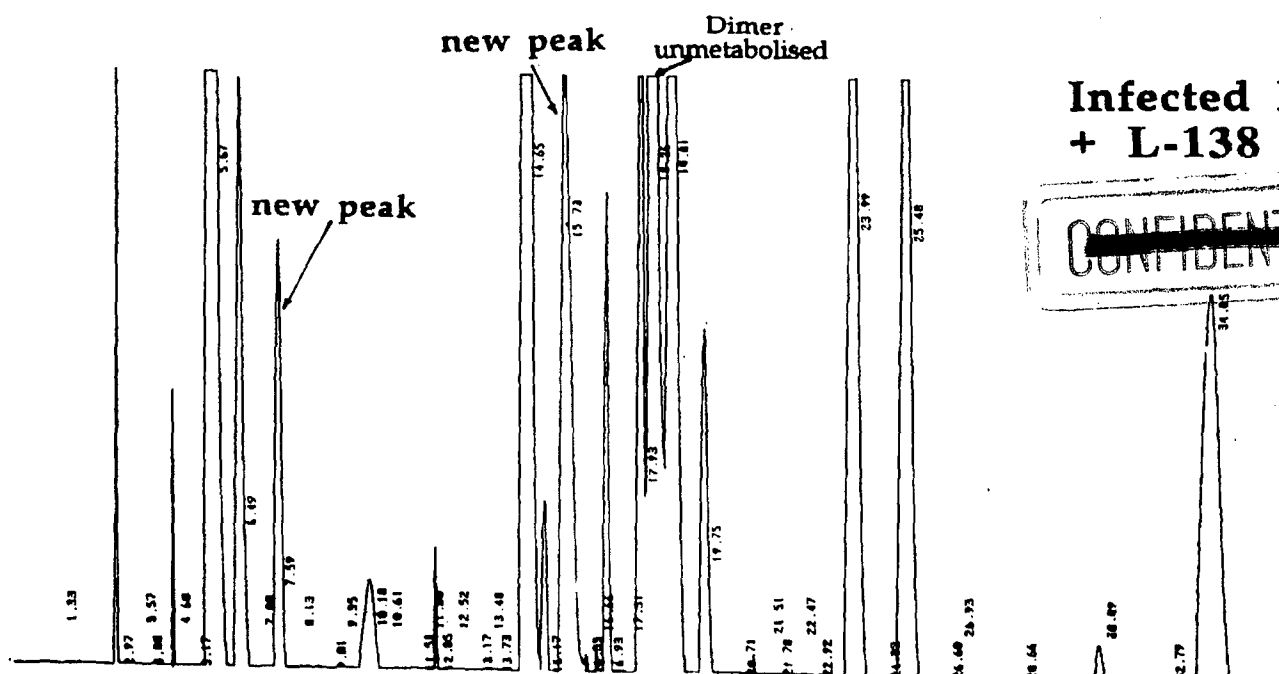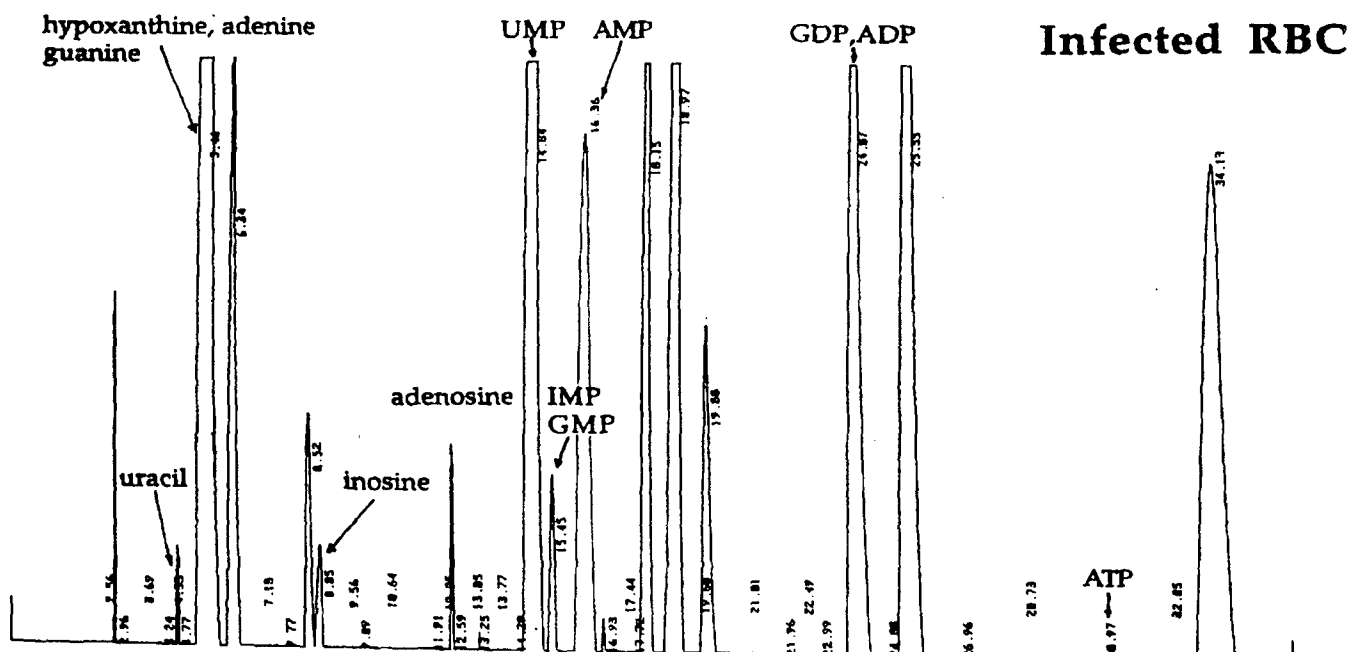| SUBMITTER REQUESTER	SUBM CODE	WRNO	BN	QOH	PARASITE	IC50	EXP DATE	CTRL DRUG CTRL IC50	CHEMICAL NAME
GOROVITS, D	L-117	281021	BN87019	0	D6	2350.889	27-MAR-97	CHLOROQUIN 2.517	BETA-L-DU,BETA-D-FUDR
GOROVITS, D	L-117	281021	BN87019	0	D6	686.374	21-APR-97	CHLOROQUIN 3.830	BETA-L-DU,BETA-D-FUDR
GOROVITS, D	L-117	281021	BN87019	0	W2	466.486	27-MAR-97	CHLOROQUIN 55.043	BETA-L-DU,BETA-D-FUDR
GOROVITS, D	L-117	281021	BN87019	0	W2	2325.683	21-APR-97	CHLOROQUIN 5.023	BETA-L-DU,BETA-D-FUDR
GOROVITS, D	L-133	281022	BN87064	0	D6	3261.496	27-MAR-97	CHLOROQUIN 2.517	BETA-L-DG,BETA-D-FUDR
GOROVITS, D	L-133	281022	BN87064	0	W2	1721.176	27-MAR-97	CHLOROQUIN 55.043	BETA-L-DG,BETA-D-FUDR
GOROVITS, D	L-125	281023	BN87055	0	D6	>50000	27-MAR-97	CHLOROQUIN 2.517	ALPHA-L-DA,BETA-D-FUDR
GOROVITS, D	L-125	281023	BN87055	0	W2	>50000	27-MAR-97	CHLOROQUIN 55.043	ALPHA-L-DA,BETA-D-FUDR

X. APPENDIX 4

The results of the transport and metabolism study in normal and infected human erythrocyte for selected Lipitek's compounds provided by the laboratories of Dr. Gero
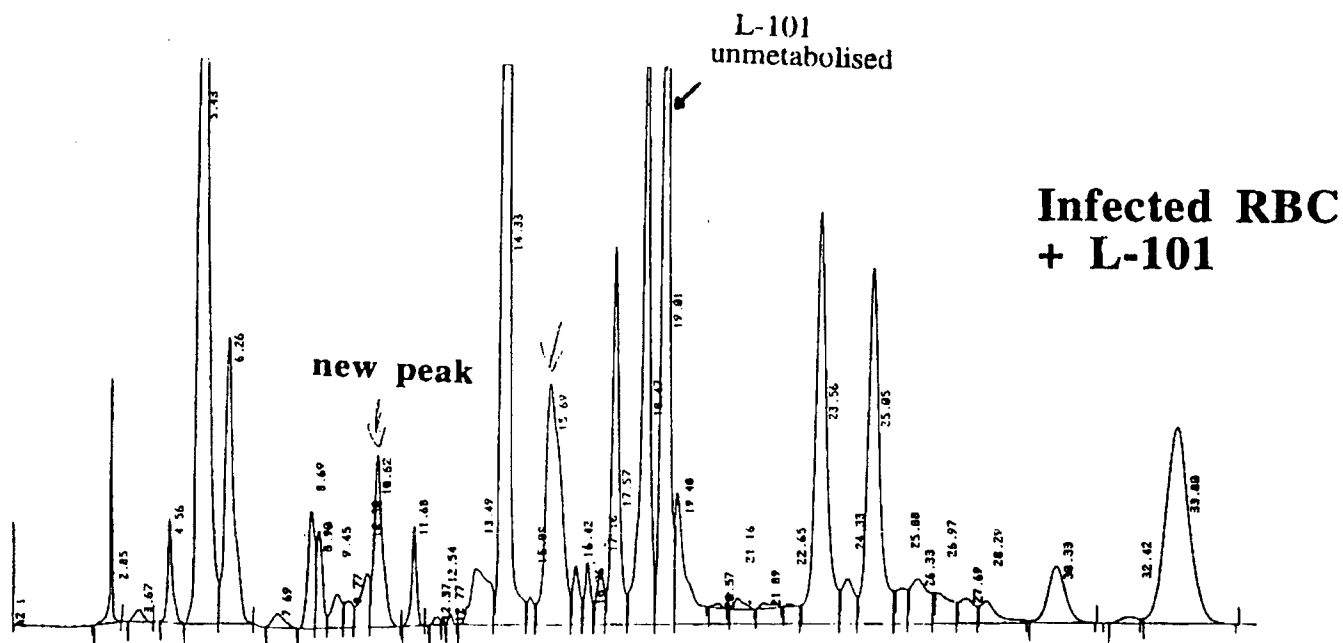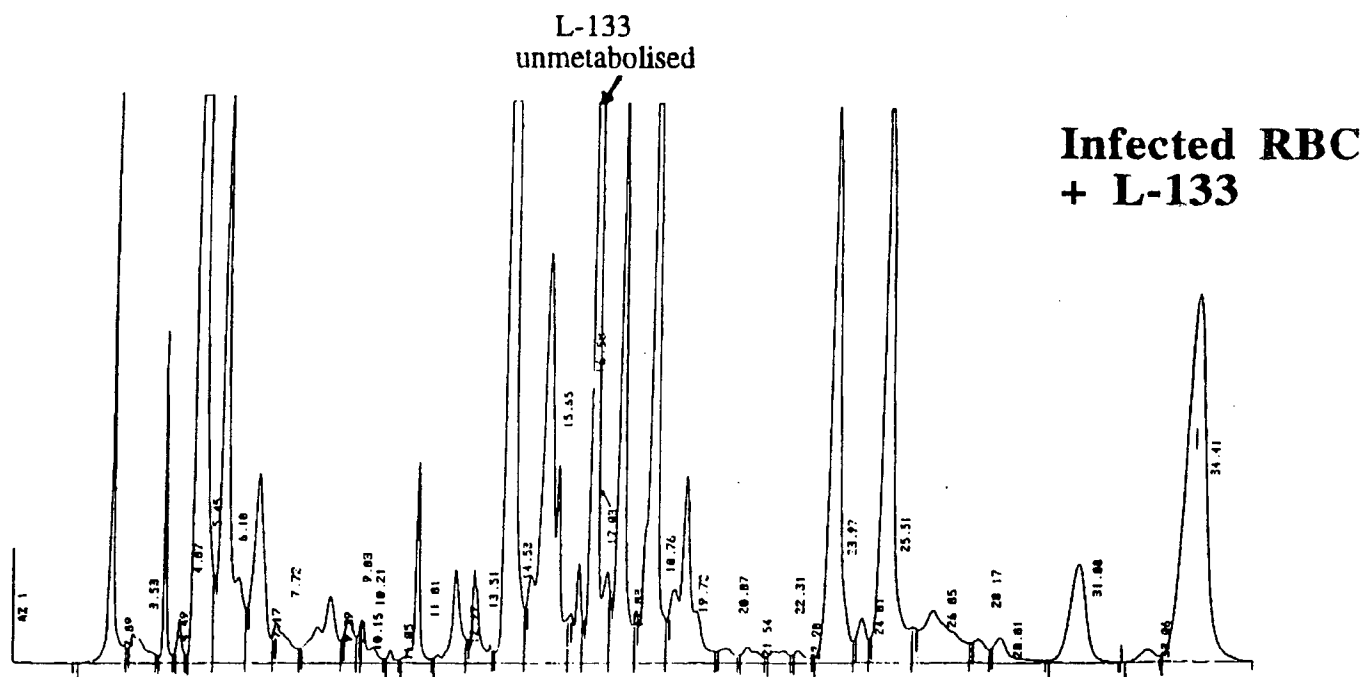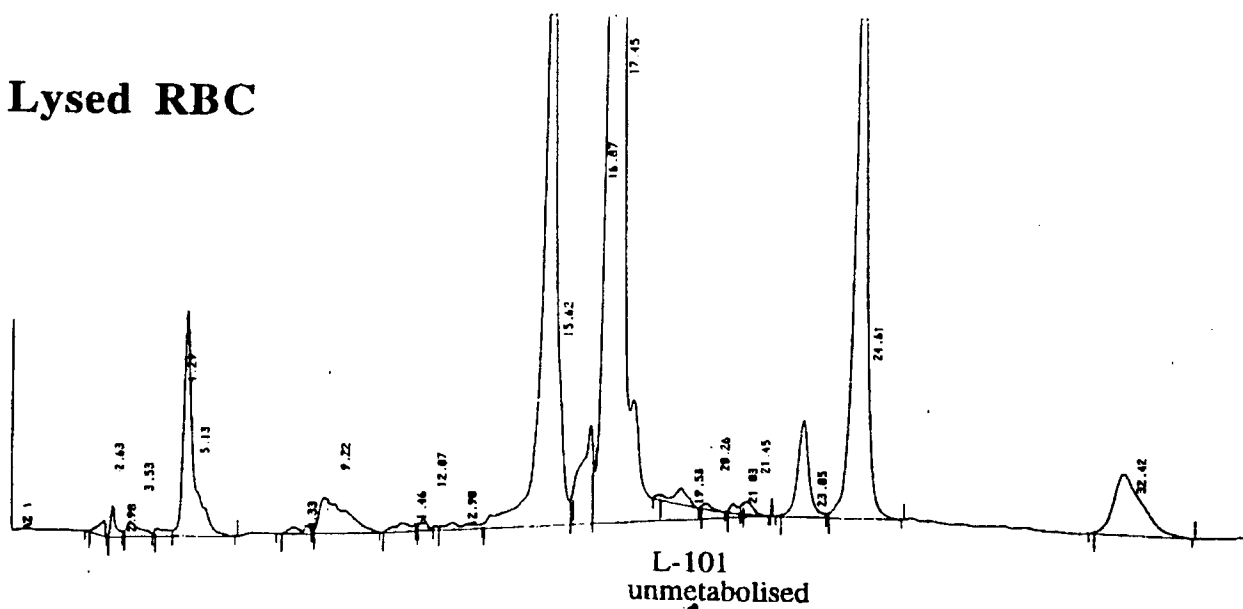
L-138

Infected RBC

hypoxanthine, adenine
guanine

UMP   AMP

GDP ADP

uracil

adenosine   IMP
GMP

inosine

ATP

**Infected RBC
+ L-138**

new peak

Dimer
unmetabolised

new peak

CONFIDENTIAL

L-133
unmetabolised

**Infected RBC
+ L-133**

**Infected RBC
+ L-101**

L-101
unmetabolised

new peak

CONFIDENTIAL

Lysed RBC

L-101
unmetabolised

**Lysed RBC
+ L-101**
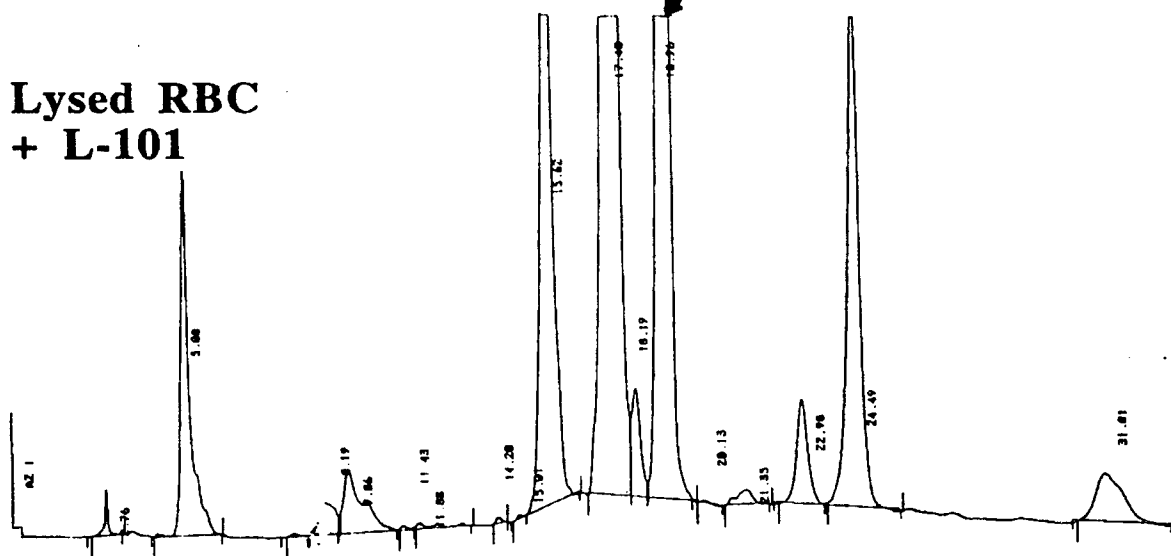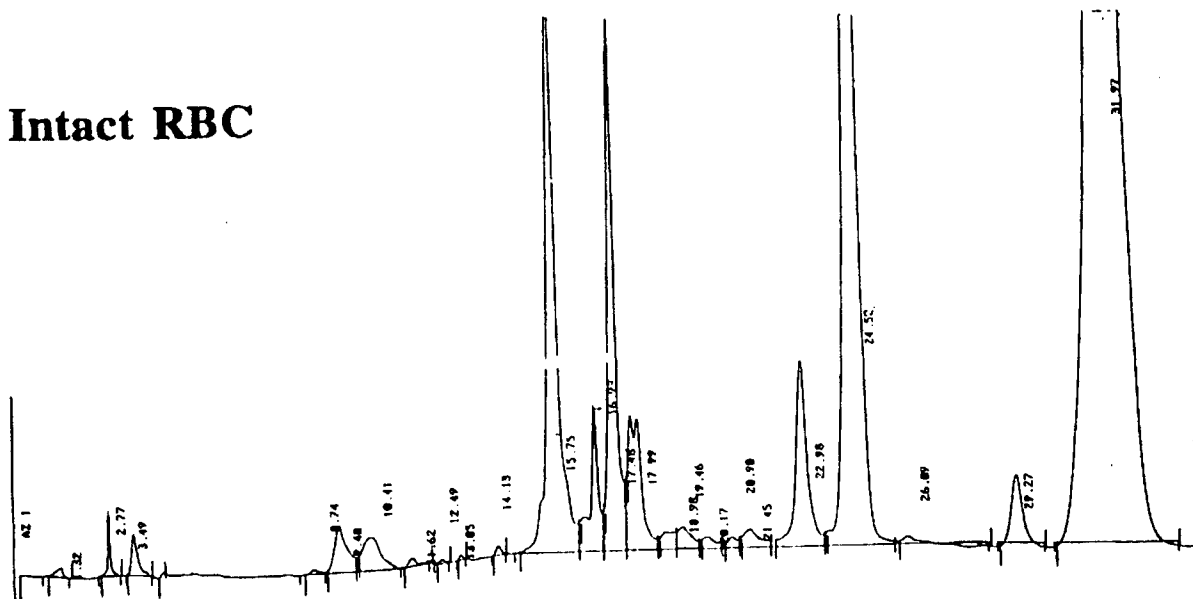
Intact RBC

Intact RBC + L-101
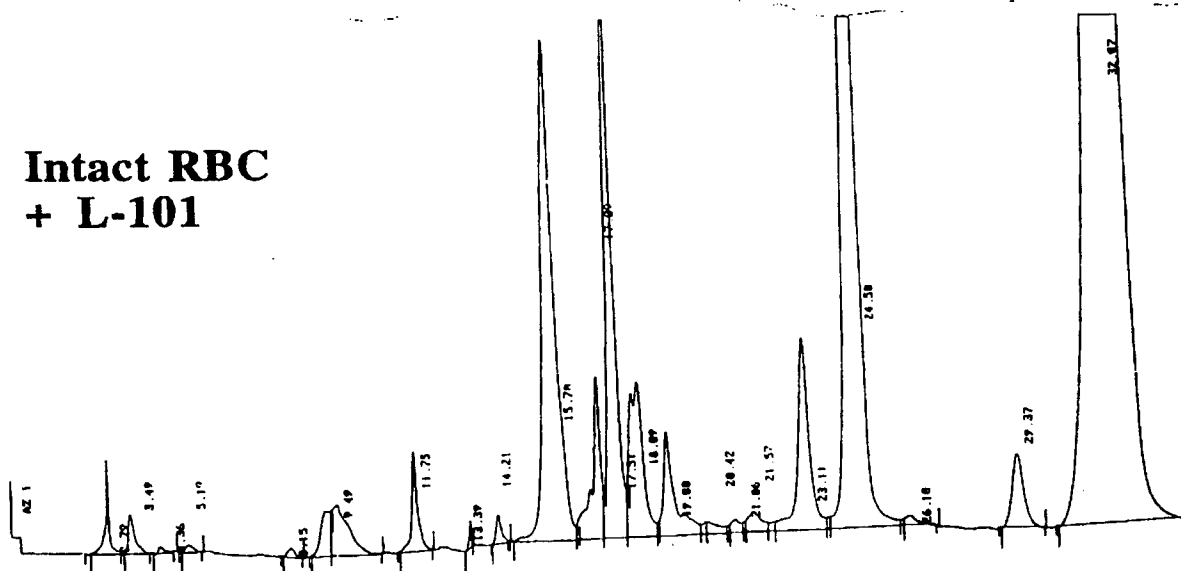
The following Lipitek International employees were partially compensated by this Phase I SBIR.

Principal Investigator   Elena Gorovits, Ph.d
Co-Principal Investigator   Charles T. Goodhue, PhD.
Senior Research Chemist   Kirupathevy Pulenthiran, Ph.D.
Senior Research Chemist   Peech Reddy, Ph.D.

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)   4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,
VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has
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These reports should be released to the National Technical
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2. Point of contact for this request is Ms. Kristin Morrow at
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FOR THE COMMANDER:

Encl   PHYLIS M. RINEHART
                                    Deputy Chief of Staff for
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